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(54) **PHARMACEUTICAL COMPOSITION FOR TREATMENT AND/OR PREVENTION OF CANCER**

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(57) **ABSTRACT**

This invention provides an antibody targeting a cancer antigenic protein specifically expressed on the surface of cancer cells and use thereof as a therapeutic and/or preventive agent for cancer. Specifically, this invention provides an antibody or a fragment thereof which has immunological reactivity with a partial CAPRIN-1 polypeptide consisting of the amino acid sequence set forth in SEQ ID NO: 5 or an amino acid sequence having 80% or higher sequence identity to the amino acid sequence, and a pharmaceutical composition for treatment and/or prevention of cancer, comprising the antibody or fragment thereof as an active ingredient.

20 Claims, No Drawings

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PHARMACEUTICAL COMPOSITION FOR TREATMENT AND/OR PREVENTION OF CANCER

TECHNICAL FIELD

The present invention relates to novel use of an antibody against CAPRIN-1 or a fragment thereof in a drug such as a therapeutic and/or preventive agent for cancer.

BACKGROUND ART

Cancer is the leading cause of death. This disease is currently treated principally by surgical therapy in combination with radiation therapy and/or chemotherapy. In spite of recent development of novel surgical techniques or discovery of novel anticancer agents, the existing treatment of cancer has an insufficiently improved outcome, except for some cancer types. With recent advances of molecular biology or cancer immunology, antibodies that specifically react with cancer, cancer antigens that are recognized by cytotoxic T cells, genes encoding such cancer antigens, and the like have been identified, raising expectations on specific cancer therapy targeting the cancer antigens (Non Patent Literature 1).

For reducing the adverse reaction of cancer therapy, it is desired that peptides, polypeptides, or proteins recognized as antigens of the cancer should rarely exist in normal cells and specifically exist in cancer cells. In 1991, Boon et al. (Ludwig Institute for Cancer Research, Belgium) isolated a human melanoma antigen MAGE1 recognized by CD8-positive T cells by a cDNA expression cloning method using autologous cancer cell lines and cancer-reactive T cells (Non Patent Literature 2). Then, a SEREX (serological identification of antigens by recombinant expression cloning) method has been reported, which adopts a gene expression cloning approach to identify tumor antigens recognized by antibodies produced in response to autologous cancer in vivo in a cancer patient (Non Patent Literature 3 and Patent Literature 1). According to this method, some cancer antigens that are rarely expressed in normal cells and are specifically expressed in cancer have been isolated (Non Patent Literatures 4 to 9). In addition, cell therapy using immunocytes that specifically react with cancer antigens or cancer-specific immunotherapy using vaccines or the like comprising cancer antigens is under clinical trial targeting some of the isolated cancer antigens.

In recent years, various antibody drugs for cancer treatment targeting antigenic proteins on cancer cells have emerged in the world. These drugs have received attention because of their certain efficacy as cancer-specific therapeutic agents. A large majority of antigenic proteins targeted by the drugs, however, are also expressed in normal cells. As a result of administering the antibodies, cancer cells as well as normal cells expressing the antigens are damaged, disadvantageously resulting in adverse reaction. Thus, if cancer antigens specifically expressed on the surface of cancer cells can be identified and antibodies targeting the antigens can be used as drugs, these antibody drugs can be expected to achieve treatment with less adverse reaction.

Cytoplasmic- and proliferation-associated protein 1 (CAPRIN-1) has been known as an intracellular protein that is expressed upon activation or cell division of resting normal cells and forms cytoplasmic stress granules with RNAs in cells to participate in the regulation of transport and translation of mRNAs. This protein has been found to be specifically expressed on the surface of cancer cells and is under study as a target of antibody drugs for cancer treatment (Patent Literature 2).

CITATION LIST

Patent Literature

Patent Literature 1: U.S. Pat. No. 5,698,396
Patent Literature 2: WO2010/016526

Non Patent Literature

Non Patent Literature 1: Tsuyoshi Akiyoshi, "Japanese Journal of Cancer and Chemotherapy", 1997, Vol. 24, p. 55-519 (Japanese Journal of Cancer and Chemotherapy Publishers Inc., Japan)
Non Patent Literature 2: Bruggen P. et al., Science, 254: 1643-1647 (1991)
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Non Patent Literature 4: Int. J. Cancer, 72: 965-971 (1997)
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SUMMARY OF INVENTION

Technical Problem

An object of the present invention is to produce an antibody that targets CAPRIN-1 specifically expressed on the surface of cancer cells and is superior in antitumor activity to conventional antibodies, and to provide use thereof as a therapeutic and/or preventive agent for cancer.

Solution to Problem

The present invention has the following aspects:

The present invention provides an antibody or a fragment thereof which has immunological reactivity with a partial CAPRIN-1 polypeptide having the amino acid sequence set forth in SEQ ID NO: 5 or an amino acid sequence having 80% or higher sequence identity to the amino acid sequence, and a pharmaceutical composition for treatment and/or prevention of cancer, comprising the antibody or fragment thereof as an active ingredient.

In an embodiment of the present invention, the cancer is breast cancer, kidney cancer, pancreatic cancer, colorectal cancer, lung cancer, brain tumor, gastric cancer, uterine cervix cancer, ovary cancer, prostate cancer, urinary bladder cancer, esophageal cancer, leukemia, lymphoma, fibrosarcoma, mastocytoma, or melanoma.

In another embodiment, the antibody is a monoclonal antibody or a polyclonal antibody.

In another embodiment, the antibody is a human antibody, a humanized antibody, a chimeric antibody, a single-chain antibody, or a multispecific antibody (e.g., a bispecific antibody).

The present specification includes the contents of Japanese Patent Application No. 2012-035342 to which the present application claims priority.

Advantageous Effects of Invention

The antibody against CAPRIN-1 according to the present invention damages cancer cells. Thus, the antibody against CAPRIN-1 is useful in the treatment or prevention of cancer.

DESCRIPTION OF EMBODIMENTS

The antibody according to the present invention is an antibody that recognizes and binds to a predetermined partial polypeptide of CAPRIN-1 and has antitumor activity. More specifically, the antibody according to the present invention is an antibody that recognizes (i.e., has immunological reactivity with) a partial polypeptide of a CAPRIN-1 protein (partial CAPRIN-1 polypeptide) consisting of the amino acid sequence set forth in SEQ ID NO: 5 or an amino acid sequence having 80% or higher, preferably 85% or higher, more preferably 90% or higher, further preferably 95% or higher sequence identity to the amino acid sequence. In the present invention, this antibody has been shown to exhibit antitumor activity. The present invention relates to all antibodies that bind to the fragments of CAPRIN-1 proteins as described above and exhibit antitumor activity.

The antibody against CAPRIN-1 according to the present invention may be any type of antibody that can exert antitumor activity and includes, for example, recombinant antibodies (e.g., synthetic antibodies, multispecific antibodies (e.g., bispecific antibodies), humanized antibodies, chimeric antibodies, and single-chain antibodies (scFv)) human antibodies, and their antibody fragments (e.g., Fab, $F(ab')_2$, and Fv). These antibodies and fragments thereof can be prepared by methods known to those skilled in the art. Desirably, the antibody according to the present invention has immunological reactivity with a CAPRIN-1 protein or a partial polypeptide thereof, i.e., binds to the CAPRIN-1 protein through antigen-antibody reaction, preferably, specifically binds to the CAPRIN-1 protein. In this context, the phrase "specifically binding to the CAPRIN-1 protein" means that the antibody specifically binds to the CAPRIN-1 protein without substantially binding to other proteins. The antibody according to the present invention is preferably a monoclonal antibody and however, may be a polyclonal antibody as long as homogeneous antibodies can be stably produced. In the case of a human subject, a human antibody or a humanized antibody is desirable for avoiding or suppressing rejection.

The antibody against a CAPRIN-1 polypeptide according to the present invention can be evaluated for its antitumor activity, as described later, by examining in vivo the inhibition of tumor growth in a cancer-bearing animal or by examining ex vivo the presence or absence of immunocyte- or complement-mediated cytotoxic activity exhibited by the antibody against tumor cells expressing the polypeptide.

The subject to receive the treatment and/or prevention of cancer according to the present invention is a mammal such as a human, a pet animal, livestock, or a sport animal and is preferably a human.

Hereinafter, the present invention will be described in more detail.

<Preparation of Antigen for Antibody Preparation>

Proteins or fragments thereof used as sensitizing antigens for obtaining the antibody against CAPRIN-1 according to the present invention are not limited by animal species serving as their origins, including humans, dogs, cats, cattle, horses, mice, rats, and chickens. The proteins or the fragments thereof, however, are preferably selected in view of compatibility with parent cells for use in cell fusion. In general, mammal-derived proteins are preferred. Particularly, human-derived proteins are preferred. For example, when CAPRIN-1 is human CAPRIN-1, human CAPRIN-1 proteins, partial peptides thereof, or cells expressing human CAPRIN-1 can be used.

The nucleotide sequences and amino acid sequences of human CAPRIN-1 and homologs thereof can be obtained, for example, by making an access to GenBank (NCBI, USA) and using an algorithm such as BLAST or FASTA (Karlin and Altschul, Proc. Natl. Acad. Sci. USA, 90: 5873-5877, 1993; and Altschul et al., Nucleic Acids Res. 25: 3389-3402, 1997).

In the present invention, with reference to the nucleotide sequence (SEQ ID NO: 1 or 3) or amino acid sequence (SEQ ID NO: 2 or 4) of human CAPRIN-1, the target CAPRIN-1 is a nucleic acid or a protein consisting of a sequence having 70% to 100%, preferably 80% to 100%, more preferably 90% to 100%, further preferably 95% to 100%, for example, 97% to 100%, 98% to 100%, 99% to 100%, or 99.5% to 100% sequence identity to the nucleotide sequence or amino acid sequence of the ORF or mature portion of the reference sequence. Note that the amino acid sequences of SEQ ID NO: 2 and SEQ ID NO: 4 compared with each other differ in amino acid residues at and following position 690. In this context, the term "% sequence identity" means a percentage (%) of the number of identical amino acids (or nucleotide bases) to the total number of amino acids (or nucleotide bases) when two sequences are aligned such that the maximum degree of similarity or identity can be achieved with or without introduced gaps.

As a CAPRIN-1 protein fragment, a fragment that comprises an epitope (or an antigenic determinant), which is the smallest unit recognized by an antibody, and has a length ranging from the amino acid length of the epitope to less than the full-length of the CAPRIN-1 protein can be used. The epitope refers to a polypeptide fragment having antigenicity or immunogenicity in mammals, preferably humans. Its smallest unit consists of approximately 7 to 12 amino acids, for example, 8 to 11 amino acids. The CAPRIN-1 protein fragment to be used in the preparation of the antibody according to the present invention is preferably a fragment that is recognized by the antibody of the present invention and comprises the amino acid sequence set forth in SEQ ID NO: 5 (which corresponds to the sequence from positions 237 to 252 in the amino acid sequence of SEQ ID NO: 2 or 4) or an amino acid sequence having 80% or higher, preferably 85% or higher, more preferably 90% or higher, further preferably 95% or higher sequence identity to the amino acid sequence, or comprises at least an epitope consisting of approximately 7 to 12 consecutive amino acids, for example, 8 to 11 consecutive amino acids in any of these amino acid sequences.

The above human CAPRIN-1 proteins and polypeptide fragments comprising partial peptides thereof can be synthesized according to chemical synthesis methods, for example, Fmoc (fluorenylmethyloxycarbonyl) and tBoc (t-butyloxycarbonyl) methods (Seikagaku Jikken Koza (Biochemical Experimentation Course in English) 1, the Japanese Biochemical Society ed., Protein Chemistry IV, Chemical Modification and Peptide Synthesis, Tokyo Kagaku Dojin Co., Ltd. (Japan), 1981). Also, these polypeptides can be synthesized by conventional methods using various commercially available peptide synthesizers.

Alternatively, polynucleotides encoding the polypeptides may be prepared using genetic engineering approaches known in the art (Sambrook et al., Molecular Cloning, the 2nd

edition, Current Protocols in Molecular Biology (1989), Cold Spring Harbor Laboratory Press; Ausubel et al., Short Protocols in Molecular Biology, the 3rd edition, A compendium of Methods from Current Protocols in Molecular Biology (1995), John Wiley & Sons; etc.) and incorporated into expression vectors, which are then introduced into host cells to produce the polypeptides produce in the host cells. In this way, the human CAPRIN-1 proteins or polypeptide fragments thereof of interest can be obtained.

The polynucleotides encoding the polypeptides can be readily prepared by genetic engineering approaches known in the art or conventional methods using commercially available nucleic acid synthesizers. For example, a DNA comprising the nucleotide sequence of a human CAPRIN-1 gene can be prepared by PCR using a human chromosomal DNA or cDNA library as a template and a pair of primers designed so as to be capable of amplifying the nucleotide sequence. Reaction conditions for this PCR can be appropriately determined Examples of the conditions can include, but not limited to, 30 cycles each involving reaction steps of 94° C. for 30 seconds (denaturation), 55° C. for 30 seconds to 1 minute (annealing), and 72° C. for 2 minutes (elongation) using thermostable DNA polymerase (e.g., Taq polymerase or Pfu polymerase) and a Mg^{2+}-containing PCR buffer, followed by reaction at 72° C. for 7 minutes. The PCR approach, conditions, etc. are described in, for example, Ausubel et al., Short Protocols in Molecular Biology, the 3rd edition, A Compendium of Methods from Current Protocols in Molecular Biology (1995), John Wiley & Sons (particularly, Chapter 15).

Also, appropriate probes or primers can be prepared on the basis of information about the nucleotide sequences of CAPRIN-1 genes and the amino acid sequences of CAPRIN-1 proteins, and used in the screening of, for example, a human cDNA library, to isolate the desired DNA. Preferably, such a cDNA library is produced from cells, organs, or tissues expressing CAPRIN-1 proteins. Examples of such cells or tissues include cells or tissues derived from the testis or from cancers or tumors such as leukemia, breast cancer, lymphoma, brain tumor, lung cancer, pancreatic cancer, and colorectal cancer. These operations, including the preparation of probes or primers, the construction of a cDNA library, the screening of the cDNA library, and the cloning of the gene of interest, are known to those skilled in the art and can be performed according to methods described in, for example, Sambrook et al., Molecular Cloning, the 2nd edition, Current Protocols in Molecular Biology (1989), and Ausubel et al. (ibid.). DNAs encoding the human CAPRIN-1 proteins and the partial peptides thereof can be obtained from the DNA thus obtained.

The host cells into which the expression vectors are introduced may be any cell capable of expressing the above polypeptides. Examples of prokaryotic cells include, but not limited to, *E. coli*. Examples of eukaryotic cells include, but not limited to: mammalian cells such as monkey kidney cells COS1 and Chinese hamster ovary cells CHO; a human embryonic kidney cell line HEK293; a mouse embryonic skin cell line NIH3T3; yeast cells such as budding yeast and fission yeast cells; silkworm cells; and *Xenopus* egg cells.

In the case of using prokaryotic cells as the host cells, the expression vectors used have an origin that permits replication in the prokaryotic cells, a promoter, a ribosomal binding site, a multicloning site, a terminator, a drug resistance gene, an auxotrophic complementary gene, etc. Examples of expression vectors for *E. coli* can include pUC series, pBluescript II, pET expression systems, and pGEX expression systems. The DNAs encoding the above polypeptides can be incorporated into such expression vectors, with which prokaryotic host cells are then transformed, followed by culture of the obtained transformants so that the polypeptides encoded by the DNAs are expressed in the prokaryotic host cells. In this respect, the polypeptides may be expressed as fusion proteins with other proteins.

In the case of using eukaryotic cells as the host cells, expression vectors for eukaryotic cells having a promoter, a splicing region, a poly(A) addition site, etc. are used as the expression vectors. Examples of such expression vectors can include pKA1, pCDM8, pSVK3, pMSG, pSVL, pBK-CMV, pBK-RSV, EBV, pRS, pcDNA3, and pYES2 vectors. In the same way as above, the DNAs encoding the above polypeptides can be incorporated into such expression vectors, with which eukaryotic host cells are then transformed, followed by culture of the obtained transformants so that the polypeptides encoded by the DNAs are expressed in the eukaryotic host cells. In the case of using expression vectors such as pINDN5-His, pFLAG-CMV-2, pEGFP-N1, or pEGFP-C1, the polypeptides may be expressed as various fusion proteins tagged with His tag (e.g., $(His)_6$ to $(His)_{10}$), FLAG tag, myc tag, HA tag, GFP, or the like.

The expression vectors can be introduced into the host cells using well known methods such as electroporation, a calcium phosphate method, a liposome method, a DEAE dextran method, microinjection, viral infection, lipofection, and binding with cell-penetrating peptides.

The polypeptide of interest can be isolated and purified from the host cells by a combination of separation operations known in the art. Examples thereof include, but not limited to, treatment with a denaturant (e.g., urea) or a surfactant, ultrasonication, enzymatic digestion, salting-out, solvent fractionation and precipitation, dialysis, centrifugation, ultrafiltration, gel filtration, SDS-PAGE, isoelectric focusing electrophoresis, ion-exchange chromatography, hydrophobic chromatography, affinity chromatography, and reverse-phase chromatography.

The antigens thus prepared can be used as sensitizing antigens as described later for producing the antibody according to the present invention.

<Structure of Antibody>

Antibodies (immunoglobulins) are usually heteromultimeric glycoproteins each comprising at least two heavy chains and two light chains. The immunoglobulins, except for IgM, are heterotetrameric glycoproteins of approximately 150 kDa each composed of two identical light (L) chains and two identical heavy (H) chains. Typically, each light chain is connected to a heavy chain via a single covalent disulfide bond, though the number of disulfide bonds between heavy chains varies among different immunoglobulin isotypes. Each of the heavy and light chains also has an intrachain disulfide bond. Each heavy chain has a variable domain (VH region) at one end, followed by a series of constant regions. Each light chain has a variable domain (VL region) at one end and has a single constant region at the other end. The light chain constant region is aligned with the first heavy chain constant region, while the light chain variable domain is aligned with the heavy chain variable domain. Particular regions called complementarity determining regions (CDRs) in the antibody variable domains exhibit specific variability and impart binding specificity to the antibody. Portions relatively conserved in the variable regions are called framework regions (FRs). The complete heavy and light chain variable domains each comprise four FRs connected via three CDRs. These three CDRs are called CDRH1, CDRH2, and CDRH3 in this order from the N-terminal side of the heavy chain. Likewise, the CDRs are called CDRL1, CDRL2, and CDRL3 in the light chain. CDRH3 is most important for the binding

specificity of the antibody for its antigen. In addition, CDRs in each chain are kept close to each other by the FR regions and contribute to the formation of an antigen-binding site in the antibody, together with CDRs in the other chain. The constant regions do not directly contribute to antibody-antigen binding, but exhibit various effector functions, for example, involvement in antibody-dependent cellular cytotoxicity (ADCC), phagocytosis mediated by binding to an Fcγ receptor, half-life/clearance rate mediated by a neonatal Fc receptor (FcRn), and complement-dependent cytotoxicity (CDC) mediated by a C1q component in the complement cascade.

<Preparation of Antibody>

The anti-CAPRIN-1 antibody according to the present invention means an antibody having immunological reactivity with a full-length CAPRIN-1 protein or a fragment thereof. Particularly, the anti-CAPRIN-1 antibody of the present invention is an antibody immunologically binding to a partial polypeptide of a CAPRIN-1 protein (partial CAPRIN-1 polypeptide) that is a peptide consisting of the epitope-containing amino acid sequence set forth in SEQ ID NO: 5 or a polypeptide consisting of an amino acid sequence having 80% or higher, preferably 85% or higher, more preferably 90% or higher, further preferably 95% or higher sequence identity to the amino acid sequence. Preferably, the antibody of the present invention recognizes an epitope consisting of approximately 7 to 12 consecutive amino acids, for example, 8 to 11 consecutive amino acids, in the amino acid sequence set forth in SEQ ID NO: 5 or an amino acid sequence having 80% or higher, preferably 85% or higher, more preferably 90% or higher, further preferably 95% or higher sequence identity to the amino acid sequence. This anti-CAPRIN-1 antibody of the present invention is capable of specifically binding to the full-length CAPRIN-1 protein. The antibody of the present invention can be obtained by selecting an antibody immunologically binding to the polypeptide consisting of the amino acid sequence set forth in SEQ ID NO: 5 or a polypeptide consisting of an amino acid sequence having 80% or higher, preferably 85% or higher, more preferably 90% or higher, further preferably 95% or higher sequence identity to the amino acid sequence, according to a routine method from among antibodies obtained with CAPRIN-1 proteins or fragments thereof as antigens.

In this context, the "immunological reactivity" means the property of the antibody binding to the CAPRIN-1 antigen (full-length CAPRIN-1 protein or partial polypeptide thereof) in vivo. Via such binding to CAPRIN-1, the antibody of the present invention exerts the function of damaging (e.g., killing, suppressing, or regressing) tumor cells. The antibody of the present invention can damage tumors, for example, breast cancer, kidney cancer, pancreatic cancer, colorectal cancer (e.g., colon cancer), lung cancer, brain tumor, gastric cancer, uterine cervix cancer, ovary cancer, prostate cancer, urinary bladder cancer, esophageal cancer, leukemia, lymphoma, fibrosarcoma, mastocytoma, or melanoma through binding to the CAPRIN-1 protein.

The antibody of the present invention may be any type of antibody. Examples of the type of the antibody of the present invention include monoclonal antibodies, polyclonal antibodies, synthetic antibodies, multispecific antibodies, human antibodies, humanized antibodies, chimeric antibodies, single-chain antibodies, and antibody fragments (e.g., Fab, $F(ab')_2$, and Fv). Also, the antibody is any class of immunoglobulin molecule, for example, IgG, IgE, IgM, IgA, IgD, or IgY, or any subclass, for example, IgG1, IgG2, IgG3, IgG4, IgA1, or IgA2.

Further, the antibody may be modified by acetylation, formylation, amidation, phosphorylation, PEGylation, or the like, as well as glycosylation.

Hereinafter, preparation examples of various antibodies will be shown.

When the antibody of the present invention is a monoclonal antibody, for example, a breast cancer cell line SK-BR-3 expressing CAPRIN-1 is administered to each mouse for immunization. The spleen is extracted from this mouse. After separation of spleen cells, the cells are fused with mouse myeloma cells. Clones producing antibodies having a cancer cell growth inhibitory effect are selected from among the obtained fusion cells (hybridomas). Alternatively, clones producing antibodies binding to a polypeptide consisting of the amino acid sequence set forth in SEQ ID NO: 5 or a polypeptide consisting of an amino acid sequence having 80% or higher sequence identity to the amino acid sequence may be selected. The hybridomas producing monoclonal antibodies having a cancer cell growth inhibitory effect or the hybridomas producing monoclonal antibodies against the polypeptide of SEQ ID NO: 5 or the like are isolated and cultured. The antibody of the present invention can be prepared by purification from the culture supernatant according to a general affinity purification method.

The monoclonal antibody-producing hybridomas may be prepared, for example, as follows. First, animals are immunized with sensitizing antigens according to a method known in the art. This immunization method generally involves intraperitoneally or subcutaneously injecting the sensitizing antigens to mammals. Specifically, the sensitizing antigens are diluted with or suspended in PBS (phosphate-buffered saline), physiological saline, or the like into an appropriate amount and then mixed, if desired, with an appropriate amount of a conventional adjuvant, for example, a complete Freund's adjuvant. After emulsification, the resulting emulsion is administered to each mammal several times every 4 to 21 days. Alternatively, an appropriate carrier may be used for the immunization with sensitizing antigens.

After confirmation of a rise in the level of the desired antibody in the serum of the mammal thus immunized, immunocytes are collected from the mammal and subjected to cell fusion. Preferred examples of the immunocytes particularly include spleen cells.

Mammalian myeloma cells are used as partner parent cells to be fused with the immunocytes. Various cell lines known in the art, for example, P3U1 (P3-X63Ag8U1), P3 (P3x63Ag8.653) (J. Immunol. (1979) 123, 1548-1550), P3x63Ag8U.1 (Current Topics in Microbiology and Immunology (1978) 81, 1-7), NS-1 (Kohler. G. and Milstein, C. Eur. J. Immunol. (1976) 6, 511-519), MPC-11 (Margulies. D. H. et al., Cell (1976) 8, 405-415), SP2/0 (Shulman, M. et al., Nature (1978) 276, 269-270), FO (deSt. Groth, S. F. et al., J. Immunol. Methods (1980) 35, 1-21), 5194 (Trowbridge, I. S. J. Exp. Med. (1978) 148, 313-323), and R210 (Galfre, G. et al., Nature (1979) 277, 131-133), are preferably used as the myeloma cells.

The cell fusion between the immunocytes and the myeloma cells can be performed basically according to a method known in the art, for example, the method of Kohler and Milstein (Kohler, G. and Milstein, C. Methods Enzymol. (1981) 73, 3-46).

More specifically, the cell fusion is carried out, for example, in the presence of a cell fusion promoter in a conventional nutrient medium. For example, polyethylene glycol (PEG) or hemagglutinating virus of Japan (HVJ) is used as

the fusion promoter. If desired, an auxiliary such as dimethyl sulfoxide may be further added for use in order to enhance fusion efficiency.

The ratio between the immunocytes and the myeloma cells used can be arbitrarily set. For example, the amount of the immunocytes is preferably set to 1 to 10 times the amount of the myeloma cells. Examples of the medium that can be used in the cell fusion include RPMI1640 and MEM media suitable for the growth of the myeloma cell lines as well as conventional media for use in this type of cell culture. In addition, a serum supplement such as fetal calf serum (FCS) may be used in combination with these media.

For the cell fusion, the immunocytes and the myeloma cells are well mixed in a predetermined amount of the medium. A PEG solution (average molecular weight: for example, approximately 1000 to 6000) preheated to approximately 37° C. is usually added to the mixture at a concentration of 30 to 60% (w/v) and mixed therewith to form the hybridomas of interest. Subsequently, procedures of sequentially adding an appropriate medium and removing the supernatant by centrifugation are preferably repeated to remove cell fusion agents or the like unfavorable for the growth of the hybridomas.

The hybridomas thus obtained are cultured in a conventional selection medium, for example, a HAT medium (medium containing hypoxanthine, aminopterin, and thymidine) for selection. This culture in the HAT medium is continued for a period (usually, several days to several weeks) sufficient for the death of cells (non-fused cells) other than the hybridomas of interest. Subsequently, hybridomas producing the antibody of interest are screened for and cloned as single clones by a conventional limiting dilution method.

In addition to such obtainment of the hybridomas by the immunization of non-human animals with antigens, hybridomas producing human antibodies having the desired activity (e.g., cell growth inhibitory activity) may be obtained by sensitizing human lymphocytes, for example, EB virus-infected human lymphocytes, with proteins, protein-expressing cells, or lysates thereof in vitro and fusing the sensitized lymphocytes with human-derived myeloma cells capable of dividing permanently, for example, U266 (Accession No. TIB196).

The monoclonal antibody-producing hybridomas thus prepared can be subcultured in a conventional medium and can also be stored for a long period in liquid nitrogen.

Specifically, the desired antigens or cells expressing the desired antigens are used as sensitizing antigens in immunization according to a conventional immunization method. The obtained immunocytes are fused with parent cells known in the art according to a conventional cell fusion method. Monoclonal antibody-producing cells (hybridomas) can be screened for by a conventional screening method to prepare monoclonal antibody-producing hybridomas against the desired antigens.

Another example of the antibody that may be used in the present invention is a polyclonal antibody. The polyclonal antibody can be obtained, for example, as follows:

Serum is obtained from small animals such as mice, human antibody-producing mice, or rabbits immunized with natural CAPRIN-1 proteins or recombinant CAPRIN-1 proteins expressed as fusion proteins with GST or the like in microorganisms such as *E. coli*, or partial peptides thereof. Alternatively, serum may be obtained from mammals immunized with CAPRIN-1 fragments as sensitizing antigens, each being a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 5 or an amino acid sequence having 80% or higher, preferably 85% or higher, more preferably 90% or higher, further preferably 95% or higher sequence identity to the amino acid sequence (preferably, a polypeptide consisting of any of these amino acid sequences), or a polypeptide comprising an epitope (preferably, consisting of the epitope) consisting of approximately 7 to 12 consecutive amino acids, for example, 8 to 11 consecutive amino acids, in the amino acid sequence set forth in SEQ ID NO: 5 or an amino acid sequence having 80% or higher, preferably 85% or higher, more preferably 90% or higher, further preferably 95% or higher sequence identity to the amino acid sequence. The serum thus obtained can be purified using, for example, ammonium sulfate precipitation, protein A or protein G columns, DEAE ion-exchange chromatography, or affinity columns coupled with CAPRIN-1 proteins or synthetic peptides to prepare anti-CAPRIN-1 polyclonal antibodies. The polyclonal antibody of the present invention includes antibodies obtained from human antibody-producing animals (e.g., mice) immunized with CAPRIN-1 proteins.

In this context, for example, KM mice (Kirin Pharma Co., Ltd./Medarex) and Xeno mice (Amgen Inc.) are known as the human antibody-producing mice (e.g., International Publication Nos. WO02/43478 and WO02/092812). Complete human polyclonal antibodies can be obtained from the blood of such mice immunized with CAPRIN-1 proteins or fragments thereof. Alternatively, spleen cells may be isolated from the mice thus immunized and fused with myeloma cells. In this way, human monoclonal antibodies can be obtained.

The antigens can be prepared according to, for example, a method using animal cells (JP Patent Publication (Kohyo) No. 2007-530068 A (2007)) or a method using baculovirus (e.g., International Publication No. WO98/46777). Antigens having low immunogenicity can be bound to immunogenic macromolecules such as albumin for immunization. The antigens may be administered together with adjuvants for immunization.

Alternatively, the antibody of the present invention may be obtained as a gene recombinant antibody that is produced using a genetic engineering technique which involves: cloning antibody genes from hybridomas; incorporating the antibody genes into appropriate vectors; and introducing the vectors into hosts (see, e.g., Carl, A. K. Borrebaeck, James, W. Larrick, THERAPEUTIC MONOCLONAL ANTIBODIES, Published in the United Kingdom by MACMILLAN PUBLISHERS LTD, 1990). Specifically, antibody variable region (V region) cDNAs are synthesized from the mRNAs of hybridomas using reverse transcriptase. After obtainment of DNAs encoding the antibody V regions of interest, the DNAs are ligated with DNAs encoding the desired antibody constant regions (C regions). The resulting ligation products are incorporated into expression vectors. Alternatively, the antibody V region-encoding DNAs may be incorporated into expression vectors containing antibody C region DNAs. These DNAs are incorporated into the expression vectors so as to be expressed under the control of expression control regions, for example, an enhancer and a promoter. Next, host cells can be transformed with the resulting expression vectors and allowed to express antibodies.

The anti-CAPRIN-1 antibody of the present invention is preferably a monoclonal antibody. Alternatively, the anti-CAPRIN-1 antibody of the present invention may be a polyclonal antibody, a genetically engineered antibody (chimeric antibody, humanized antibody, etc.), or the like.

The monoclonal antibody includes human monoclonal antibodies, non-human animal monoclonal antibodies (e.g., mouse, rat, rabbit, and chicken monoclonal antibodies), chimeric monoclonal antibodies, and the like. The monoclonal antibody may be prepared by the culture of hybridomas

obtained by the fusion between spleen cells from non-human mammals (e.g., mice, human antibody-producing mice, chickens, or rabbits) immunized with CAPRIN-1 proteins or fragments thereof and myeloma cells. The chimeric antibody is an antibody prepared from a combination of sequences derived from different animals and is, for example, an antibody composed of mouse antibody heavy and light chain variable regions and human antibody heavy and light chain constant regions. The chimeric antibody can be prepared using a method known in the art which involves, for example: ligating DNAs encoding mouse antibody V regions with DNAs encoding human antibody C regions; incorporating the resulting ligation products into expression vectors; and introducing the vectors into hosts so that antibodies are produced.

Monoclonal antibodies that have immunological reactivity with the partial CAPRIN-1 polypeptide consisting of the amino acid sequence set forth in SEQ ID NO: 5 and have an antitumor effect are prepared by the method disclosed in Examples described below.

The humanized antibody, also called reshaped human antibody, is an engineered antibody. The humanized antibody is constructed by grafting antibody CDRs derived from an immunized animal into complementarity determining regions of a human antibody. A general gene recombination approach therefor is also known.

Specifically, for example, DNA sequences designed so as to link mouse, rabbit, or chicken antibody CDRs, and human antibody framework regions (FRs) are synthesized by PCR using several prepared oligonucleotides having terminal portions overlapping with each other. The obtained DNAs are ligated with DNAs encoding human antibody constant regions. Subsequently, the resulting ligation products are incorporated into expression vectors, which are then introduced into hosts for antibody production to obtain the antibody of interest (see European Patent Application Publication No. EP239400 and International Publication No. WO96/02576). The human antibody FRs connected via CDRs are selected such that the complementarity determining regions form a favorable antigen-binding site. If necessary, amino acids in the framework regions of antibody variable regions may be substituted such that the complementarity determining regions of the resulting reshaped human antibody form an appropriate antigen-binding site (Sato K. et al., Cancer Research 1993, 53: 851-856). In addition, the FRs may be replaced with framework regions derived from human antibodies of different class or subclass (see International Publication No. WO99/51743).

The human antibody framework regions connected via CDRs are selected such that the complementarity determining regions form a favorable antigen-binding site. If necessary, amino acids in the framework regions of antibody variable regions may be substituted such that the complementarity determining regions of the resulting reshaped human antibody form an appropriate antigen-binding site (Sato K. et al., Cancer Research 1993, 53: 851-856).

Amino acids in variable regions (e.g., FRs) or constant regions of the chimeric antibody or the humanized antibody thus prepared may be substituted, for example, by other amino acids.

The amino acid substitution is the substitution of, for example, less than 15, less than 10, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less amino acids, preferably 1 to 5 amino acids, more preferably 1 or 2 amino acids. The substituted antibody should be functionally equivalent to an unsubstituted antibody. The substitution is desirably conservative amino acid substitution, which is the substitution between amino acids similar in properties such as charge, side chains, polarity, and aromaticity. The amino acids can be classified in terms of similar properties into, for example: basic amino acids (arginine, lysine, and histidine); acidic amino acids (aspartic acid and glutamic acid); uncharged polar amino acids (glycine, asparagine, glutamine, serine, threonine, cysteine, and tyrosine); nonpolar amino acids (leucine, isoleucine, alanine, valine, proline, phenylalanine, tryptophan, and methionine); branched amino acids (leucine, valine, and isoleucine); and aromatic amino acids (phenylalanine, tyrosine, tryptophan, and histidine).

Examples of modified antibodies can include antibodies bound with various molecules such as polyethylene glycol (PEG). In the modified antibody of the present invention, the substance to be bound is not limited. Such a modified antibody can be obtained by chemically modifying the obtained antibody. A method therefor has already been established in the art.

In this context, the phrase "functionally equivalent" means that an antibody concerned has biological or biochemical activity similar to that of the antibody of the present invention, specifically, the antibody concerned has the function of damaging tumor and essentially causes no rejection when applied to humans, for example. Examples of such activity can include cell growth inhibitory activity and binding activity.

A method for preparing a polypeptide functionally equivalent to a certain polypeptide, which involves introducing a mutation into a polypeptide, is well known to those skilled in the art. For example, those skilled in the art can introduce a mutation as appropriate into the antibody of the present invention using site-directed mutagenesis (Hashimoto-Gotoh, T. et al., (1995) Gene 152, 271-275; Zoller, M J., and Smith, M. (1983) Methods Enzymol. 100, 468-500; Kramer, W. et al., (1984) Nucleic Acids Res. 12, 9441-9456; Kramer, W. and Fritz, H J., (1987) Methods Enzymol. 154, 350-367; Kunkel, T A., (1985) Proc. Natl. Acad. Sci. USA. 82, 488-492; and Kunkel (1988) Methods Enzymol. 85, 2763-2766) or the like, thereby preparing an antibody functionally equivalent to the antibody of the present invention.

The above-mentioned antibody that recognizes an epitope of a CAPRIN-1 protein or a CAPRIN-1 fragment polypeptide comprising the epitope can be obtained by a method known to those skilled in the art. For example, the antibody can be obtained by a method which involves determining the epitope of the CAPRIN-1 protein recognized by the obtained anti-CAPRIN-1 antibody having a cancer cell growth inhibitory effect by a conventional method (e.g., epitope mapping or an epitope identification method described later) and preparing an antibody using a polypeptide having an amino acid sequence contained in the epitope as an immunogen, or a method which involves determining an epitope for an antibody prepared by a conventional method and selecting an antibody that recognizes the same epitope as that for the anti-CAPRIN-1 antibody. In this context, the "epitope" refers to a polypeptide fragment having antigenicity or immunogenicity in mammals, preferably humans. Its smallest unit consists of approximately 7 to 12 amino acids, preferably 8 to 11 amino acids.

The antibody of the present invention is an antibody having immunological reactivity with CAPRIN-1, an antibody that specifically recognizes CAPRIN-1, or an antibody that specifically binds to CAPRIN-1 and exhibits cytotoxic activity against cancer or a tumor growth inhibitory effect. The antibody is preferably an antibody having a structure that causes little or no rejection in recipient animals. Examples of such antibodies include human antibodies, humanized antibodies, chimeric antibodies (e.g., human-mouse chimeric antibodies), single-chain antibodies, and bispecific antibodies when

the recipient animals are humans. These antibodies have heavy and light chain variable regions derived from a human antibody or have heavy and light chain variable regions with complementarity determining regions (CDR1, CDR2, and CDR3) derived from a non-human animal antibody and framework regions (FR1, FR2, FR3, and FR4) derived from a human antibody. Alternatively, these antibodies are recombinant antibodies having heavy and light chain variable regions derived from a non-human animal antibody and heavy and light chain constant regions derived from a human antibody. The antibody of the present invention is preferably the former two antibodies.

Such recombinant antibodies can be prepared as follows: DNAs encoding monoclonal antibodies (e.g., human, mouse, rat, rabbit, and chicken monoclonal antibodies) against human CAPRIN-1 are cloned from antibody-producing cells such as hybridomas and used as templates in RT-PCR or the like to prepare DNAs encoding the light and heavy chain variable regions of the antibodies. The respective sequences of the light and heavy chain variable regions, the respective sequences of CDR1, CDR2, and CDR3 in each region, or the respective sequences of FR1, FR2, FR3, and FR4 in each region can be determined on the basis of the Kabat EU numbering system (Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institute of Health, Bethesda, Md. (1991)).

Such a DNA encoding each variable region or a DNA encoding each CDR is prepared using a genetic engineering technique (Sambrook et al., Molecular Cloning A Laboratory Manual, Cold Spring Harbor Laboratory Press (1989)) or a DNA synthesizer. In this context, the human monoclonal antibody-producing hybridomas can be prepared by immunizing human antibody-producing animals (e.g., mice) with human CAPRIN-1 and then fusing spleen cells excised from the immunized animals with myeloma cells. Aside from this, DNAs encoding human antibody-derived light or heavy chain variable and constant regions are prepared, if necessary, using a genetic engineering technique or a DNA synthesizer.

For the humanized antibody, DNAs in which the CDR coding sequences in DNAs encoding human antibody-derived light or heavy chain variable regions are substituted by corresponding CDR coding sequences of a non-human animal (e.g., mouse, rat, rabbit, or chicken)-derived antibody can be prepared and ligated with the DNAs encoding human antibody-derived light or heavy chain constant regions to prepare a DNA encoding the humanized antibody.

For the chimeric antibody, DNAs encoding light or heavy chain variable regions of a non-human animal (e.g., mouse, rat, rabbit, or chicken)-derived antibody can be ligated with DNAs encoding human antibody-derived light or heavy chain constant regions to prepare a DNA encoding the chimeric antibody.

The single-chain antibody refers to an antibody comprising heavy and light chain variable regions linearly linked to each other via a linker. A DNA encoding the single-chain antibody can be prepared by ligating a DNA encoding the heavy chain variable region, a DNA encoding the linker, and a DNA encoding the light chain variable region. In this context, the heavy and light chain variable regions are both derived from a human antibody or derived from a human antibody in which CDRs alone are substituted by CDRs of a non-human animal (e.g., mouse, rat, rabbit, or chicken)-derived antibody. The linker consists of 12 to 19 amino acids. Examples thereof include $(G_4S)_3$ consisting of 15 amino acids (G. B. Kim et al., Protein Engineering Design and Selection 2007, 20 (9): 425-432).

The bispecific antibody (e.g., diabody) refers to an antibody capable of specifically binding to two different epitopes. A DNA encoding the bispecific antibody can be prepared by, for example, ligating a DNA encoding a heavy chain variable region A, a DNA encoding a light chain variable region B, a DNA encoding a heavy chain variable region B, and a DNA encoding a light chain variable region A in this order (provided that the DNA encoding a light chain variable region B and the DNA encoding a heavy chain variable region B are ligated via a DNA encoding a linker as described above). In this context, the heavy and light chain variable regions are all derived from a human antibody or derived from a human antibody in which CDRs alone are substituted by CDRs of a non-human animal (e.g., mouse, rat, rabbit, or chicken)-derived antibody.

The recombinant DNAs thus prepared can be incorporated into one or more appropriate vectors, which are then introduced into host cells (e.g., mammalian cells, yeast cells, and insect cells) and the DNAs are (co)expressed to produce recombinant antibodies (P. J. Delves., ANTIBODY PRODUCTION ESSENTIAL TECHNIQUES., 1997 WILEY, P. Shepherd and C. Dean., Monoclonal Antibodies., 2000 OXFORD UNIVERSITY PRESS; and J. W. Goding., Monoclonal Antibodies: principles and practice., 1993 ACADEMIC PRESS).

Examples of the antibody of the present invention prepared by any of the methods described above include the following antibody (a):

(a) an antibody comprising a heavy chain variable region comprising complementarity determining regions of SEQ ID NOs: 8, 9, and 10 and a light chain variable region comprising complementarity determining regions of SEQ ID NOs: 12, 13, and 14 (e.g., an antibody comprising a heavy chain variable region of SEQ ID NO: 11 and a light chain variable region of SEQ ID NO: 15); and

Herein, the amino acid sequences shown by SEQ ID NOs: 8, 9, and 10 correspond to heavy chain variable regions CDR1, CDR2, and CDR3, respectively. The amino acid sequences shown by SEQ ID NOs: 12, 13, and 14 correspond to light chain variable regions CDR1, CDR2, and CDR3, respectively.

Examples of the humanized antibody, the chimeric antibody, the single-chain antibody, or the bispecific antibody of the present invention include the following antibodies (i) to (iii):

(i) an antibody comprising a heavy chain variable region comprising CDR1, CDR2, and CDR3 consisting of the amino acid sequences of SEQ ID NOs: 8, 9, and 10, respectively, and human antibody-derived framework regions and a light chain variable region comprising CDR1, CDR2, and CDR3 consisting of the amino acid sequences of SEQ ID NOs: 12, 13, and 14, respectively, and human antibody-derived framework regions;

(ii) an antibody comprising a heavy chain comprising a heavy chain variable region comprising CDR1, CDR2, and CDR3 consisting of the amino acid sequences of SEQ ID NOs: 8, 9, and 10, respectively, and human antibody-derived framework regions, and a human antibody-derived heavy chain constant region, and a light chain comprising a light chain variable region comprising CDR1, CDR2, and CDR3 consisting of the amino acid sequences of SEQ ID NOs: 12, 13, and 14, respectively, and human antibody-derived framework regions, and a human antibody-derived a light chain constant region; and

(iii) an antibody comprising a heavy chain comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 11 and a human antibody-derived

heavy chain constant region, and a light chain comprising a light chain variable region comprising the amino acid sequence of SEQ ID NO: 15 and a human antibody-derived light chain constant region.

The sequences of the constant and variable regions of human antibody heavy and light chains are available from, for example, NCBI (USA; GenBank, UniGene, etc.). For example, the following sequences can be referred to: Accession No. J00228 for a human IgG1 heavy chain constant region; Accession No. J00230 for a human IgG2 heavy chain constant region; Accession No. X03604 for a human IgG3 heavy chain constant region; Accession No. K01316 for a human IgG4 heavy chain constant region; Accession Nos. V00557, X64135, X64133, etc. for a human light chain κ constant region; and Accession Nos. X64132, X64134, etc. for a human light chain λ, constant region.

Preferably, these antibodies have cytotoxic activity and can thereby exert an antitumor effect.

The above particular sequences of the heavy and light chain variable regions and CDRs in each antibody are provided merely for illustrative purposes. It is clear that the antibody of the present invention is not limited by the particular sequences. Hybridomas capable of producing anti-human CAPRIN-1 human antibodies or non-human animal antibodies (e.g., mouse antibodies) different from those described above can be prepared, and monoclonal antibodies produced by the hybridomas can be recovered and determined as being (or being not) the antibodies of interest using immunological binding activity against human CAPRIN-1 and cytotoxic activity as indicators. The monoclonal antibody-producing hybridomas of interest are thereby identified. Then, DNAs encoding the heavy and light chain variable regions of the antibodies of interest are produced from the hybridomas and sequenced, as described above. The DNAs can be used for the preparation of different antibodies.

The above antibodies may each have the substitution, deletion, or addition of one or several amino acids, particularly in a framework region sequence and/or a constant region sequence, as long as the antibody has such specificity that it can specifically recognize CAPRIN-1. In this context, the term "several" means preferably 2 to 5, more preferably 2 or 3.

The affinity constant Ka (k_{on}/k_{off}) of the antibody of the present invention for a CAPRIN-1 protein or a fragment thereof is preferably at least 10^7 M^{-1}, at least 10^8 M^{-1}, at least 5×10^8 M^{-1}, at least 10^9 M^{-1}, at least 5×10^9 M^{-1}, at least 10^{10} M^{-1}, at least 5×10^{10} M^{-1}, at least 10^{11} M^{-1}, at least 5×10^{11} M^{-1}, at least 10^{12} M^{-1}, or at least 10^{13} M^{-1}.

The antibody of the present invention can be conjugated with an antitumor agent. The conjugation of the antibody with the antitumor agent can be performed via a spacer having a group reactive with an amino group, a carboxyl group, a hydroxy group, a thiol group, or the like (e.g., a succinimidyl group, a formyl group, a 2-pyridyldithio group, a maleimidyl group, an alkoxycarbonyl group, or a hydroxy group).

Examples of the antitumor agent include the following antitumor agents known by literatures, etc.: paclitaxel, doxorubicin, daunorubicin, cyclophosphamide, methotrexate, 5-fluorouracil, thiotepa, busulfan, improsulfan, piposulfan, benzodopa, carboquone, meturedopa, uredopa, altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide, trimethylolomelamine, bullatacin, bullatacinone, camptothecin, bryostatin, callystatin, cryptophycin 1, cryptophycin 8, dolastatin, duocarmycin, eleutherobin, pancratistatin, sarcodictyin, spongistatin, chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard, carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine, calicheamicin, dynemicin, clodronate, esperamicin, aclacinomycin, actinomycin, authramycin, azaserine, bleomycin, cactinomycin, carabicin, carminomyc in, carzinophilin, chromomycin, dactinomycin, detorbicin, 6-diazo-5-oxo-L-norleucine, Adriamycin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycin C, mycophenolic acid, nogalamycin, olivomycin, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin, denopterin, pteropterin, trimetrexate, fludarabine, 6-mercaptopurine, thiamiprine, thioguanine, ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, androgens (e.g., calusterone, dromostanolone propionate, epitiostanol, mepitiostane, and testolactone), aminoglutethimide, mitotane, trilostane, frolinic acid, aceglatone, aldophosphamide glycoside, aminolevulinic acid, eniluracil, amsacrine, bestrabucil, bisantrene, edatraxate, defofamine, demecolcine, diaziquone, elfornithine, elliptinium acetate, epothilone, etoglucid, lentinan, lonidamine, maytansine, ansamitocin, mitoguazone, mitoxantrone, mopidanmol, nitraerine, pentostatin, phenamet, pirarubicin, losoxantrone, podophyllinic acid, 2-ethylhydrazide, procarbazine, razoxane, rhizoxin, schizophyllan, spirogermanium, tenuazonic acid, triaziquone, roridin A, anguidine, urethane, vindesine, dacarbazine, mannomustine, mitobronitol, mitolactol, pipobroman, gacytosine, docetaxel, chlorambucil, gemcitabine, 6-thioguanine, mercaptopurine, cisplatin, oxaliplatin, carboplatin, vinblastine, etoposide, ifosfamide, mitoxantrone, vincristine, vinorelbine, novantrone, teniposide, edatrexate, daunomycin, aminopterin, Xeloda, ibandronate, irinotecan, topoisomerase inhibitors, difluoromethylornithine (DMFO), retinoic acid, capecitabine, and pharmaceutically acceptable salts and derivatives thereof

Alternatively, the antibody of the present invention can be administered in combination with an antitumor agent to produce a higher therapeutic effect. This approach is adaptable to a patient with cancer expressing CAPRIN-1 either before or after surgical operation. This approach can be applied, particularly after surgery, to CAPRIN-1-expressing cancer, which has been treated conventionally with an antitumor agent alone, to produce higher prevention of cancer recurrence or prolongation of survival time.

Examples of the antitumor agent used in the combined administration with the antibody of the present invention include the following antitumor agents known by literatures, etc.: paclitaxel, doxorubicin, daunorubicin, cyclophosphamide, methotrexate, 5-fluorouracil, thiotepa, busulfan, improsulfan, piposulfan, benzodopa, carboquone, meturedopa, uredopa, altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide, trimethylolomelamine, bullatacin, bullatacinone, camptothecin, bryostatin, callystatin, cryptophycin 1, cryptophycin 8, dolastatin, duocarmycin, eleutherobin, pancratistatin, sarcodictyin, spongistatin, chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard, carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine, calicheamicin, dynemicin, clodronate, esperamicin, aclacinomycin, actinomycin, authramycin, azaserine, bleomycin, cactinomycin, carabicin, carminomycin, carzinophilin, chromomycin, dactinomycin, detorbicin, 6-diazo-5-oxo-L-norleucine, Adriamycin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycin C,

mycophenolic acid, nogalamycin, olivomycin, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin, denopterin, pteropterin, trimetrexate, fludarabine, 6-mercaptopurine, thiamiprine, thioguanine, ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone, aminoglutethimide, mitotane, trilostane, frolinic acid, aceglatone, aldophosphamide glycoside, aminolevulinic acid, eniluracil, amsacrine, bestrabucil, bisantrene, edatraxate, defofamine, demecolcine, diaziquone, elfornithine, elliptinium acetate, epothilone, etoglucid, lentinan, lonidamine, maytansine, ansamitocin, mitoguazone, mitoxantrone, mopidanmol, nitraerine, pentostatin, phenamet, pirarubicin, losoxantrone, podophyllinic acid, 2-ethylhydrazide, procarbazine, razoxane, rhizoxin, schizophyllan, spirogermanium, tenuazonic acid, triaziquone, roridin A, anguidine, urethane, vindesine, dacarbazine, mannomustine, mitobronitol, mitolactol, pipobroman, gacytosine, docetaxel, chlorambucil, gemcitabine, 6-thioguanine, mercaptopurine, cisplatin, oxaliplatin, carboplatin, vinblastine, etoposide, ifosfamide, mitoxantrone, vincristine, vinorelbine, novantrone, teniposide, edatrexate, daunomycin, aminopterin, Xeloda, ibandronate, irinotecan, topoisomerase inhibitors, difluoromethylornithine (DMFO), retinoic acid, capecitabine, and pharmaceutically acceptable salts (known in the art) and derivatives (known in the art) thereof. Of these antitumor agents, cyclophosphamide, paclitaxel, docetaxel, or vinorelbine is particularly preferably used.

The antibody of the present invention may be bound to a radioisotope known by literatures, etc., such as ^{211}At, ^{131}I, ^{125}I, ^{90}Re, ^{186}Re, ^{153}Sm, ^{212}Bi, ^{32}P, ^{175}Lu, ^{176}Lu, ^{89}Sr, ^{64}Cu, or ^{111}In (Hideo Saji, YAKUGAKU ZASSHI 128 (3) 323-332, 8 (2008), Jpn). A radioisotope effective for the treatment or diagnosis of tumor is desirable. Such a radioisotope is also included in the antitumor agent according to the present invention.

<Identification of Epitope>

As shown in Examples below, the antibody of the present invention binds to an epitope in the amino acid sequence set forth in SEQ ID NO: 5. One example of a method for confirming an epitope for the antibody of the present invention includes a method which involves immobilizing an epitope in the polypeptide of SEQ ID NO: 5 onto a plate and evaluating the antibody for its reactivity with this epitope. Specifically, an epitope in the polypeptide of SEQ ID NO: 5 is immobilized onto a plate through reaction with an electrophilic functional groups via a spacer such as oligoethylene glycol. The antibody of the present invention can be reacted with the plate and examined for its reactivity with the epitope through reaction with a labeled (e.g., horseradish peroxidase (HRP)-labeled) secondary antibody binding to the antibody of the present invention (i.e., thereby confirming the epitope to which the antibody of the present invention binds). As the epitope in the polypeptide of SEQ ID NO: 5 to be immobilized onto a plate, a sequence itself comprising at least the epitope in the sequence of SEQ ID NO: 5 or a modified portion thereof is used (e.g., N-terminal or C-terminal residues modified with several any amino acids or a protein such as KLH or a (poly) peptide modified with a MAP protein). The antibody of the present invention needs only to bind to any of these (poly) peptides.

On the other hand, some antibodies of the present invention may not react with the polypeptide of SEQ ID NO: 5, i.e., the epitope may not be confirmed, in the above method. In this case, the epitope for the antibody of the present invention can be confirmed by reacting the antibody with the antigen under solution conditions that facilitate antigen-antibody binding, obtaining the resulting antigen-antibody complex by an immunoprecipitation method, and then separating a partial polypeptide bound with the antibody, and analyzing its amino acid sequence. In this context, the antigen may be, for example, the polypeptide of SEQ ID NO: 5 itself or a modified portion thereof. Alternatively, even a CAPRIN-1 protein may be used as long as the epitope reactive with the antibody of the present invention can be confirmed by the above method.

<Antitumor Effect>

The antitumor effect of the anti-CAPRIN-1 antibody used in the present invention on CAPRIN-1-expressing cancer cells seems to be brought about by the following mechanism: effector cell-mediated antibody-dependent cellular cytotoxicity (ADCC) against the CAPRIN-1-expressing cells and complement-dependent cytotoxicity (CDC) against the CAPRIN-1-expressing cells. However, the mechanism is not intended to limit the scope of the present invention.

The antitumor effect based on the mechanism is known to correlate with the number of target molecules expressed on the surface of cancer cells to which the antibody binds (Niwa R., Clinical Cancer Research 2005 Mar. 15; 11 (6): 2327-2336). The number of target molecules expressed on the surface of cancer cells can be examined using an existing assay kit capable of measuring the number of cell surface molecules. Specifically, the number of target molecules to which the antibody binds can be determined by: reacting primary antibodies such as antibodies against the target molecules with cancer cells; reacting therewith fluorescently labeled antibodies against the primary antibodies together with beads for a calibration curve with the known number of molecules; measuring the mean fluorescence intensity of the samples and preparing a calibration curve.

Thus, the anti-CAPRIN-1 antibody to be used in the present invention can be examined for its activity, as specifically shown in Examples below, by assaying the ADCC or CDC activity against CAPRIN-1-expressing cancer cells ex vivo or by determining the number of CAPRIN-1 molecules expressed on the surface of cancer cells when using the anti-CAPRIN-1 antibody according to the present invention as a primary antibody.

The anti-CAPRIN-1 antibody to be used in the present invention binds to a CAPRIN-1 protein on cancer cells and exhibits an antitumor effect through the activity. Thus, the anti-CAPRIN-1 antibody of the present invention is considered to be useful in the treatment or prevention of cancer. Specifically, the present invention provides a pharmaceutical composition for treatment and/or prevention of cancer, comprising the anti-CAPRIN-1 antibody as an active ingredient. The anti-CAPRIN-1 antibody to be used for the purpose of administration to human bodies (antibody therapy) is preferably a human antibody or a humanized antibody for reducing immunogenicity.

An anti-CAPRIN-1 antibody with higher binding affinity for a CAPRIN-1 protein on cancer cell surface exerts stronger antitumor activity. Thus, the antibody of the present invention has high binding affinity for the CAPRIN-1 protein and can therefore be expected to have a stronger antitumor effect. Accordingly, the antibody of the present invention is adaptable to a pharmaceutical composition intended for the treatment and/or prevention of cancer. The antibody of the present invention has high binding affinity with an association constant (affinity constant) Ka (k_{on}/k_{off}), of preferably at least 10^7 M^{-1}, at least 10^8 M^{-1}, at least 5×10^8 M^{-}, at least 10^9 M^{-1}, at least 5×10^9 M^{-1}, at least 10^{10} M^{-1}, at least 5×10^{10} M^{-1}, at

least 10^{11} M^{-1}, at least 5×10^{11} M^{-1}, at least 10^{12} M^{-1}, or at least 10^{13} M^{-1}, as described above.

A larger number of CAPRIN-1 molecules that can bind to anti-CAPRIN-1 antibodies on cancer cell surface produces stronger antitumor activity. Desirably, for the purpose of the expectation of the antitumor effect, the number of such CAPRIN-1 molecules is 10^4 or more, preferably 10^5 or more, per cancer cell to which the antibody binds, as determined using the anti-CAPRIN-1 antibody of the present invention. Tumor (cancer cells) having a large number of CAPRIN-1 molecules on the cell surface is particularly preferred as cancer to receive the antibody of the present invention.

<Binding to Antigen-Expressing Cell>

The ability of the antibody to bind to CAPRIN-1 can be determined by use of binding assay using, for example, ELISA assay, Western blot, immunofluorescence, and flow cytometry analysis, as described in Examples.

<Immunohistochemical Staining>

The antibody that recognizes CAPRIN-1 can be tested for its reactivity with CAPRIN-1 by an immunohistochemical method well known to those skilled in the art using a paraformaldehyde- or acetone-fixed frozen section or paraformaldehyde-fixed paraffin-embedded section of a tissue obtained from a patient during surgical operation or from an animal carrying a xenograft tissue inoculated with a cell line expressing CAPRIN-1 either spontaneously or after transfection.

For immunohistochemical staining, the antibody reactive with CAPRIN-1 can be stained by various methods. For example, the antibody can be visualized through reaction with a horseradish peroxidase-conjugated goat anti-mouse antibody, goat anti-rabbit antibody, or goat anti-chicken antibody.

<Pharmaceutical Composition and Method for Treating and/or Preventing Cancer>

A target of the pharmaceutical composition for treatment and/or prevention of cancer of the present invention is not particularly limited as long as the target is cancer (cells) expressing a CAPRIN-1 gene.

The terms "tumor" and "cancer" used herein mean malignant neoplasm and are used interchangeably with each other.

The cancer targeted in the present invention is cancer expressing a a CAPRIN-1 protein-encoding gene and is preferably breast cancer, kidney cancer, pancreatic cancer, colorectal cancer, lung cancer, brain tumor, gastric cancer, uterine cervix cancer, ovary cancer, prostate cancer, urinary bladder cancer, esophageal cancer, leukemia, lymphoma, fibrosarcoma, mastocytoma, or melanoma.

Specific examples of these cancers include, but not limited to, breast adenocarcinoma, complex-type breast adenocarcinoma, malignant mixed tumor of mammary gland, intraductal papillary adenocarcinoma, lung adenocarcinoma, squamous cell cancer, small-cell cancer, large-cell cancer, glioma which is tumor of neuroepithelial tissue, ependymoma, neuronal tumor, embryonal neuroectodermal tumor, neurilemmoma, neurofibroma, meningioma, chronic lymphocytic leukemia, lymphoma, gastrointestinal lymphoma, alimentary lymphoma, small to medium cell-type lymphoma, cecal cancer, ascending colon cancer, descending colon cancer, transverse colon cancer, sigmoid colon cancer, rectal cancer, epithelial ovarian cancer, germ cell tumor, stromal cell tumor, pancreatic ductal carcinoma, invasive pancreatic ductal carcinoma, pancreatic adenocarcinoma, acinar cell carcinoma, adenosquamous carcinoma, giant cell tumor, intraductal papillary-mucinous neoplasm, mucinous cystic neoplasm, pancreatoblastoma, serous cystadenocarcinoma, solid papillary tumor, gastrinoma, glucagonoma, insulinoma, multiple endocrine neoplasia type-1 (Wermer's syndrome), nonfunctional islet cell tumor, somatostatinoma, and VIPoma.

The recipient subjects (patients) are preferably mammals, for example, mammals including primates, pet animals, livestock, and sport animals and are particularly preferably humans, dogs, and cats.

In the case of using the antibody of the present invention in a pharmaceutical composition, the pharmaceutical composition can be formulated by a method known to those skilled in the art. For example, the pharmaceutical composition can be used in the form of a parenteral injection of an aseptic solution or suspension with water or any other pharmaceutically acceptable liquid. For example, the pharmaceutical composition may be formulated with the antibody mixed in a unit dosage form required for generally accepted pharmaceutical practice, in combination with pharmacologically acceptable carriers or media, specifically, sterilized water, physiological saline, plant oil, an emulsifier, a suspending agent, a surfactant, a stabilizer, a flavoring agent, an excipient, a vehicle, a preservative, a binder, etc., as appropriate. The amount of the active ingredient in such a preparation is determined such that an appropriate dose within the indicated range can be achieved.

An aseptic composition for injection can be formulated according to conventional pharmaceutical practice using a vehicle such as injectable distilled water.

Examples of aqueous solutions for injection include physiological saline, isotonic solutions containing glucose and other adjuvants, for example, D-sorbitol, D-mannose, D-mannitol, and sodium chloride. These solutions may be used in combination with an appropriate solubilizer, for example, an alcohol (specifically, ethanol) or a polyalcohol (e.g., propylene glycol and polyethylene glycol), or a nonionic surfactant, for example, polysorbate 80 (TM) or HCO-60.

Examples of oily solutions include sesame oil and soybean oil. The solutions may be used in combination with benzyl benzoate or benzyl alcohol as a solubilizer. The solutions may be further mixed with a buffer (e.g., a phosphate buffer solution and a sodium acetate buffer solution), a soothing agent (e.g., procaine hydrochloride), a stabilizer (e.g., benzyl alcohol and phenol), or an antioxidant. The injection solutions thus prepared are usually charged into appropriate ampules.

The pharmaceutical composition of the present invention is administered orally or parenterally, preferably parenterally. Specific examples of its dosage forms include injections, intranasal administration agents, transpulmonary administration agents, and percutaneous administration agents. Examples of the injections include intravenous injection, intramuscular injection, intraperitoneal injection, and subcutaneous injection, through which the pharmaceutical composition can be administered systemically or locally.

Also, the administration method can be appropriately selected depending on the age, weight, sex, symptoms, etc. of a patient. The dose of a pharmaceutical composition containing the antibody or a polynucleotide encoding the antibody can be selected within a range of, for example, 0.0001 to 1000 mg/kg of body weight per dose. Alternatively, the dose can be selected within a range of, for example, 0.001 to 100000 mg/body of a patient, though the dose is not necessarily limited to these numeric values. Although the dose and the administration method vary depending on the weight, age, sex, symptoms, etc. of a patient, those skilled in the art can appropriately select the dose and the method.

The pharmaceutical composition comprising the antibody or fragment thereof of the present invention can be administered to a subject to treat and/or prevent cancer, preferably

breast cancer, kidney cancer, pancreatic cancer, colorectal cancer, lung cancer, brain tumor, gastric cancer, uterine cervix cancer, ovary cancer, prostate cancer, urinary bladder cancer, esophageal cancer, leukemia, lymphoma, fibrosarcoma, mastocytoma, or melanoma.

The present invention further encompasses a method for treating and/or preventing cancer, comprising administering the pharmaceutical composition of the present invention in combination with the antitumor agent as exemplified above or a pharmaceutical composition comprising the antitumor agent to a subject. The antibody of the present invention or the fragment thereof may be administered simultaneously with or separately from the antitumor agent to the subject. In the case of separately administering these pharmaceutical compositions, either one may be administered first or later. Their dosing intervals, doses, administration routes, and the number of doses can be appropriately selected by a specialist. The other pharmaceutical dosage forms to be administered simultaneously also include, for example, pharmaceutical compositions each formulated by mixing the antibody of the present invention or the fragment thereof or the antitumor agent into a pharmacologically acceptable carrier (or medium). An instruction describing prescription, formulation, administration routes, doses, cancer, etc. as to the pharmaceutical compositions and dosage forms containing the antibody of the present invention can also be attached to any of the above-described pharmaceutical compositions and dosage forms containing the antitumor agent.

Thus, the present invention also provides a pharmaceutical combination for treatment and/or prevention of cancer, comprising the pharmaceutical composition of the present invention and a pharmaceutical composition comprising the antitumor agent as exemplified above, and a method for treating and/or preventing cancer, comprising administering the pharmaceutical combination. The present invention also provides a pharmaceutical composition for treatment and/or prevention of cancer, comprising the antibody or the fragment thereof of the present invention and the antitumor agent together with a pharmacologically acceptable carrier.

<Polypeptide and DNA>

The present invention further provides a DNA encoding the antibody of the present invention or the fragment (antibody-binding fragment) thereof. Such a DNA may be a DNA encoding the heavy and/or light chains of the antibody or may be a DNA encoding the heavy and/or light chain variable regions of the antibody. The DNA may also be a DNA encoding each or a combination of the complementarity determining regions of the antibody. The DNA includes, for example, a heavy chain variable region-encoding DNA comprising nucleotide sequences encoding the amino acid sequences of SEQ ID NOs: 8, 9, and 10 and a light chain variable region-encoding DNA comprising nucleotide sequences encoding the amino acid sequences of SEQ ID NOs: 12, 13, and 14, in the case of the antibody (a).

The complementarity determining regions (CDRs) encoded by the DNA having these sequences serve as regions that determine the specificity of the antibody. Sequences encoding the other regions (i.e., constant regions and framework regions) of the antibody may therefore be sequences derived from other antibodies. In this context, "other antibodies" also include antibodies derived from non-human organisms, but are preferably those derived from humans from the viewpoint of reducing adverse reactions. Specifically, in the DNA described above, regions encoding each framework region and each constant region in the heavy and light chains preferably comprise nucleotide sequences encoding corresponding human antibody-derived amino acid sequences.

Further examples of the DNA encoding the antibody of the present invention include a DNA comprising a nucleotide sequence encoding a heavy chain variable region comprising a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 11, and a DNA comprising a nucleotide sequence encoding a light chain variable region comprising a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 15, in the case of the antibody (a). In this context, the nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 11 is, for example, the nucleotide sequence of SEQ ID NO: 16. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 15 is, for example, the nucleotide sequence of SEQ ID NO: 17.

When such a DNA comprises a region encoding each constant region in the heavy and light chains, the region preferably comprises a nucleotide sequence encoding a corresponding human antibody-derived amino acid sequence (amino acid sequence of each constant region in the heavy and light chains).

These antibody DNAs can be obtained, for example, by the methods described above or the following method. First, total RNAs are prepared from hybridomas producing the antibody of the present invention using a commercially available RNA extraction kit, and cDNAs are synthesized therefrom using reverse transcriptase and random primers or the like. Subsequently, the antibody-encoding cDNAs are amplified by PCR using oligonucleotide primers for conserved sequences of each variable region in known mouse antibody heavy and light chain genes. Sequences encoding the constant regions can be obtained by PCR amplification of known sequences. The nucleotide sequence of the DNA can be incorporated into a plasmid or a phage for sequencing, for example, and determined according to a conventional method.

The present invention further provides the following polypeptides and DNAs related to the antibody (a):

(i) a polypeptide comprising the amino acid sequence of SEQ ID NO: 11, and a DNA encoding the polypeptide (e.g., a DNA comprising the nucleotide sequence of SEQ ID NO: 16);

(ii) a polypeptide comprising the amino acid sequence of SEQ ID NO: 15, and a DNA encoding the polypeptide (e.g., a DNA comprising the nucleotide sequence of SEQ ID NO: 17);

(iii) a heavy chain CDR polypeptide selected from the group consisting of the amino acid sequences shown by SEQ ID NOs: 8, 9, and 10, and a DNA encoding the polypeptide; and

(iv) a light chain CDR polypeptide selected from the group consisting of the amino acid sequences shown by SEQ ID NOs: 12, 13, and 14, and a DNA encoding the polypeptide.

These polypeptides and DNAs can be prepared using genetic engineering techniques as described above.

<Summary of the Present Invention>

The aspects of the present invention described above are summarized below.

(1) An antibody or a fragment thereof which has immunological reactivity with a partial CAPRIN-1 polypeptide consisting of the amino acid sequence set forth in SEQ ID NO: 5 or an amino acid sequence having 80% or higher sequence identity to the amino acid sequence.

(2) The antibody or fragment thereof according to (1), wherein the antibody or fragment thereof has cytotoxic activity against a cancer cell expressing a CAPRIN-1 protein.

(3) The antibody or fragment thereof according to (1) or (2), wherein the antibody is a monoclonal antibody or a polyclonal antibody.

(4) The antibody or fragment thereof according to any of (1) to (3), wherein the antibody is a human antibody, a humanized antibody, a chimeric antibody, a single-chain antibody, or a multispecific antibody.

(5) The antibody or fragment thereof according to any of (1) to (4), wherein the antibody or fragment thereof comprises a heavy chain variable region comprising complementarity determining regions of SEQ ID NOs: 8, 9, and 10 (CDR1, CDR2, and CDR3, respectively) and a light chain variable region comprising complementarity determining regions of SEQ ID NOs: 12, 13, and 14 (CDR1, CDR2, and CDR3, respectively) and has immunological reactivity with the CAPRIN-1 protein.

(6) The antibody or fragment thereof according to any of (1) to (5), wherein the antibody or fragment thereof is conjugated with an antitumor agent.

(7) A pharmaceutical composition for treatment and/or prevention of cancer, comprising an antibody or fragment thereof according to any of (1) to (6) as an active ingredient.

(8) The pharmaceutical composition according to (7), wherein the cancer is breast cancer, kidney cancer, pancreatic cancer, colorectal cancer, lung cancer, brain tumor, gastric cancer, uterine cervix cancer, ovary cancer, prostate cancer, urinary bladder cancer, esophageal cancer, leukemia, lymphoma, fibrosarcoma, mastocytoma, or melanoma.

(9) A pharmaceutical combination for treatment and/or prevention of cancer, comprising the pharmaceutical composition according to (7) or (8) and a pharmaceutical composition comprising an antitumor agent.

(10) A DNA encoding the antibody or fragment thereof according to any of (1) to (5).

(11) A method for treating and/or preventing cancer, comprising administering the antibody or fragment thereof according to any of (1) to (6), the pharmaceutical composition according to (7) or (8), or the pharmaceutical combination according to (9) to a subject.

EXAMPLES

Hereinafter, the present invention will be described more specifically with reference to Examples. However, the scope of the present invention is not intended to be limited by these specific examples.

Example 1

Analysis of CAPRIN-1 Expression in Each Tissue

CAPRIN-1 gene expression in canine and human normal tissues and various cell lines was examined by RT-PCR according to Example 1(4) of WO2010/016526. As a result, its strong expression was shown in the testis among the healthy canine tissues, whereas the expression was shown in canine breast cancer and adenocarcinoma tissues. As a result of also examining the expression in human tissues, the expression was observed only in the testis among normal tissues, as with the canine CAPRIN-1 gene. By contrast, concerning cancer cells, the expression was detected in many types of cancer cell lines, including 8 human breast cancer cell lines (ZR75-1, MCF7, T47D, SK-BR-3, MDA-MB-157, BT-20, MDA-MB-231V, and MRK-nu-1) and 4 pancreatic cancer cell lines (Capan-2, MIAPaCa-2, Panc-1, and BxPc-3). These results demonstrated that CAPRIN-1 is expressed in the breast cancer cell lines and the pancreatic cancer cell lines, whereas its expression is not detected in normal tissues other than the testis.

Example 2

Preparation of Mouse Monoclonal Antibody Against CAPRIN-1

(1) Preparation of Mouse Monoclonal Antibody

100 μg of a human CAPRIN-1 protein having the amino acid sequence of SEQ ID NO: 2 as prepared in Example 3 of WO2010/016526 was mixed with an equal amount of MPL+TDM adjuvant (manufactured by Sigma-Aldrich Corp.). This mixture was used as an antigen solution per mouse. The antigen solution was intraperitoneally administered to each 6-week-old Balb/c mouse (manufactured by Japan SLC, Inc.). Then, 7 boosters were performed every 1 week to complete immunization. Three days after the final administration, the spleen of each mouse was excised and ground between two sterilized glass slides. Washing with PBS(−) (manufactured by Nissui Pharmaceutical Co., Ltd.), centrifuging at 1500 rpm for 10 minutes and removing a supernatant were repeated three times to obtain spleen cells. The obtained spleen cells were mixed with mouse myeloma cells SP2/0 (purchased from ATCC) at a ratio of 10:1. 200 μl of an RPMI1640 medium containing 10% FBS was heated to 37° C. and mixed with 800 μl of PEG1500 (manufactured by Boehringer Ingelheim GmbH), and the PEG solution thus prepared was added to the cell mixture, which was then left standing for 5 minutes for cell fusion. After centrifugation at 1700 rpm for 5 minutes and removal of the supernatant, the cells were suspended in 150 ml of an RPMI1640 medium containing 15% FBS supplemented with 2% equivalent of a HAT solution (Gibco (HAT selection medium). This suspension was inoculated to fifteen 96-well plates (Nunc) at 100 μl/well. The spleen cells and the myeloma cells were fused by culture for 7 days at 37° C., 5% CO_2 to obtain hybridomas.

The resulting hybridomas were screened for the binding affinity of antibodies produced by the hybridomas against the CAPRIN-1 protein as an indicator. 1 μg/ml solution of the CAPRIN-1 protein prepared by the approach described in Example 3 of WO2010/016526 was added to a 96-well plate at 100 μl/well and left standing at 4° C. for 18 hours. Each well was washed three times with PBS-T. Then, a 0.5% bovine serum albumin (BSA) solution (manufactured by Sigma-Aldrich Corp.) was added thereto at 400 μl/well and left standing at room temperature for 3 hours. The solution in each well was discarded, and each well was washed three times with 400 μl of PBS-T. Then, the culture supernatant of each hybridoma obtained above was added thereto at 100 μl/well and left standing at room temperature for 2 hours. Each well was washed three times with PBS-T. Then, HRP-labeled anti-mouse IgG (H+L) antibodies (manufactured by Invitrogen Corp.) were added thereto at 100 μl/well of a dilution of 5000-fold with PBS and left standing at room temperature for 1 hour. Each well was washed three times with PBS-T. Then, a TMB substrate solution (manufactured by Thermo Fisher Scientific Inc.) was added thereto at 100 μl/well and left standing for 15 to 30 minutes to develop color reaction. After the color development, the reaction was terminated by the addition of 1N sulfuric acid at 100 μl/well. The absorbance was measured at 450 nm and 595 nm using an absorption spectrometer. As a result, several hybridomas producing antibodies having high absorbance were selected.

The selected hybridomas were added to a 96-well plate at 0.5 cells/well and cultured in the plate. One week later, hybridomas forming single colonies in the wells were observed. The cells in these wells were further cultured, and the cloned hybridomas were screened for the binding affinity of antibodies produced by the hybridomas against the CAPRIN-1 pro-

tein as an indicator. A 1 μg/ml solution of the CAPRIN-1 protein prepared by the approach described in Example 3 of WO2010/016526 was added to a 96-well plate at 100 μl/well and left standing at 4° C. for 18 hours. Each well was washed three times with PBS-T. Then, a 0.5% BSA solution was added thereto at 400 μl/well and left standing at room temperature for 3 hours. The solution in each well was discarded, and each well was washed three times with 400 μl of PBS-T. Then, the culture supernatant of each hybridoma obtained above was added thereto at 100 μl/well and left standing at room temperature for 2 hours. Each well was washed three times with PBS-T. Then, HRP-labeled anti-mouse IgG (H+L) antibodies (manufactured by Invitrogen Corp.) were added thereto at 100 μl/well of a dilution of 5000-fold with PBS and left standing at room temperature for 1 hour. Each well was washed three times with PBS-T. Then, a TMB substrate solution (manufactured by Thermo Fisher Scientific Inc.) was added thereto at 100 μl/well and left standing for 15 to 30 minutes to cause color reaction. After the color development, the reaction was terminated by the addition of 1N sulfuric acid at 100 μl/well. The absorbance was measured at 450 nm and 595 nm using an absorption spectrometer. As a result, 61 hybridoma lines producing monoclonal antibodies reactive with the CAPRIN-1 protein were obtained.

Next, these monoclonal antibodies were screened for antibodies reactive with the surface of breast cancer cells expressing CAPRIN-1. Specifically, 10^6 cells of a human breast cancer cell line MDA-MB-231V were centrifuged in a 1.5-ml microcentrifuge tube. 100 μl of the culture supernatant of each hybridoma obtained above was added thereto and left standing for 1 hour on ice. After washing with PBS, FITC-labeled goat anti-mouse IgG antibodies (manufactured by Invitrogen Corp.) diluted 500-fold with PBS containing 0.1% FBS were added thereto and left standing for 1 hour on ice. After washing with PBS, the fluorescence intensity was measured using FACSCalibur (Becton, Dickinson and Company). On the other hand, the same operation as above was performed using the serum of each untreated 6-week-old Balb/c mouse diluted 500-fold with a medium for hybridoma culture, instead of the antibodies, to prepare a control. As a result, one monoclonal antibody (anti-CAPRIN-1 antibody #1) having stronger fluorescence intensity than that of the control, i.e., reactive with the surface of the breast cancer cells, was selected.

(2) Identification of CAPRIN-1 Epitope Recognized by Anti-CAPRIN-1 Monoclonal Antibody #1

The cancer cell surface-reactive monoclonal antibodies against CAPRIN-1 (anti-CAPRIN-1 antibody #1) obtained in the section (1) were used to identify a CAPRIN-1 epitope region recognized thereby. 93 candidate peptides each consisting of 12 to 16 amino acids in the amino acid sequence of the human CAPRIN-1 protein were synthesized and each dissolved at a concentration of 1 mg/ml in DMSO.

Each peptide was dissolved at a concentration of 30 μg/ml in a 0.1 M sodium carbonate buffer solution (pH 9.6). The solution was added at 100 μl/well to a 96-well plate (Nunc, product No.: 436006) and left standing overnight at 4° C. The solution in each well was discarded, and 10 mM ethanolamine/0.1 M sodium carbonate buffer solution (PH 9.6) was added thereto at 200 μl/well and left standing at room temperature for 1 hour. Then, the solution in each well was discarded, and each well was washed twice with PBS containing 0.5% Tween 20 (PBST) to prepare a peptide-immobilized plate.

The cell culture supernatant containing the anti-CAPRIN-1 antibody #1 was added at 50 μl/well to the plate thus obtained. After shaking at room temperature for 1 hour, the solution in each well was discarded, and each well was washed three times with PBST. Next, a secondary antibody solution containing HRP-labeled anti-mouse IgG (manufactured by Invitrogen Corp.) antibodies diluted 3000- to 4000-fold with PBST was added thereto at 50 μl/well. Then, the solution in each well was discarded, and each well was washed six times with PBST.

A TMB substrate solution (manufactured by Thermo Fisher Scientific Inc.) was added thereto at 100 μl/well and left standing for 15 to 30 minutes to develop color reaction. After the color development, the reaction was terminated by the addition of 1N sulfuric acid at 100 μl/well. The absorbance was measured at 450 nm and 595 nm using an absorption spectrometer.

As a result, the polypeptide of SEQ ID NO: 5 was identified as a partial sequence of CAPRIN-1 recognized by the anti-CAPRIN-1 antibody #1 obtained in Example 2(1).

(3) Cloning of Variable Region Genes of Anti-CAPRIN-1 Antibody #1

The monoclonal antibodies obtained in Example 2(1) were analyzed for their variable region-encoding gene sequences and amino acid sequences thereof according to the method described in Example 5 of WO2010/016526. As a result, the monoclonal antibody #1 comprised a heavy chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 11 and a light chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 15. A gene sequence encoding the heavy chain variable region of the obtained monoclonal antibody #1 is shown in SEQ ID NO: 16, and a gene sequence encoding the light chain variable region of the monoclonal antibody #1 is shown in SEQ ID NO: 17.

It was also shown that: the monoclonal antibody #1 obtained in Example 2(1) comprises the heavy chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 11 and the light chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 15, wherein CDR1, CDR2, and CDR3 in the heavy chain variable region consist of the amino acid sequences shown by SEQ ID NOs: 8, 9, and 10, respectively, and CDR1, CDR2, and CDR3 in the light chain variable region consist of the amino acid sequences shown by SEQ ID NOs: 12, 13, and 14, respectively.

Example 3

Preparation of Polyclonal Antibody Against Partial CAPRIN-1 Polypeptide Present on Cancer Cell Surface

In order to obtain polyclonal antibodies against partial CAPRIN-1 polypeptides present on cancer cell surface, the polypeptide (CAPRIN-1-derived peptide shown in SEQ ID NO: 5) comprising the epitope region for the anti-CAPRIN-1 antibody #1 obtained in Example 2, a polypeptide corresponding to a region of amino acid residue numbers 50 to 98 in the human CAPRIN-1 amino acid sequence of SEQ ID NO: 2, and a polypeptide corresponding to a region of amino acid residue numbers 233 to 305 of SEQ ID NO: 2 were synthesized. Each of these peptides (1 mg) was mixed as an antigen with an equal volume of an incomplete Freund's adjuvant (IFA) solution. This mixture was subcutaneously administered to each rabbit four times every two weeks. Then, blood was collected to obtain antiserum containing each polyclonal antibody. This antiserum was further purified using a protein G carrier (manufactured by GE Healthcare Bio-Sciences Ltd.) and medium replacement with PBS was

carried out therefor to obtain polyclonal antibodies against partial CAPRIN-1 polypeptides present on cancer cell surface. In addition, the serum of a rabbit that received no antigen was purified using a protein G carrier in the same way as above and used as control antibodies.

Example 4

Analysis of CAPRIN-1 Protein Expression on Cancer Cell Membrane Surface

Next, 8 human breast cancer cell lines (ZR75-1, MCF7, T47D, SK-BR-3, MDA-MB-157, BT-20, MDA-MB-231V, and MRK-nu-1) observed to have a high level of CAPRIN-1 gene expression were examined for their expression of CAPRIN-1 proteins on the cell surface. 5×10^5 cells of each of human breast cancer cell lines that was observed to have gene expression in the above Example were centrifuged in a 1.5-ml microcentrifuge tube. 2 μg (5 μl) each of the polyclonal antibodies against the CAPRIN-1-derived peptide (SEQ ID NO: 5) prepared as described above in Example 3 and 95 μl of PBS containing 0.1% fetal bovine serum were added thereto and mixed, and left standing for 1 hour on ice. After washing with PBS, 1 μl of Alexa 488-labeled goat anti-rabbit IgG antibodies (manufactured by Invitrogen Corp.) and 98 μl of PBS containing 0.1% fetal bovine serum (FBS) were added to and mixed with the cells and left standing for 30 hours on ice. After washing with PBS, the fluorescence intensity was measured using FACSCalibur (Becton, Dickinson and Company). On the other hand, as a control, the same process as above was performed using the control antibody prepared as described above in Example 3 instead of the polyclonal antibodies against CAPRIN-1-derived peptides. As a result, all lines of the cancer cells administered with the anti-CAPRIN-1 antibodies exhibited fluorescence intensity at least 35% stronger than that of the control. This demonstrated that CAPRIN-1 proteins are expressed on the cell membrane surface of the human cancer cell lines. The rate of enhancement in fluorescence intensity above was expressed as the rate of increase in mean fluorescence intensity (MFI) in each cell line and calculated according to the following formula:

Rate of increase in mean fluorescence intensity(Rate of enhancement in fluorescence intensity)(%)= ((MFI of cells reacted with the anti-CAPRIN-1 antibodies)−(Control MFI))/(Control MFI)×100.

Also, the fluorescence intensity was measured in 2 kidney cancer cell lines (Caki-1 and Caki-2), a urinary bladder cancer cell line (T24), an ovary cancer cell line (SKOV3), 2 lung cancer cell lines (QG56 and A549), a prostate cancer cell line (PC3), a uterine cervix cancer cell line (SW756), a fibrosarcoma cell line (HT1080), 2 brain tumor cell lines (T98G and U87MG), a gastric cancer cell line (MNK28), 3 colorectal cancer cell lines (Lovo, DLD-1, and HCT-116), and 4 pancreatic cancer cell lines (Capan-2, MIAPaCa-2, Panc-1, and BxPC-3) using the same approach as above. As a result, all the cancer cells had fluorescence intensity at least 35% stronger than that of the control.

As with the results obtained above, CAPRIN-1 protein expression on cancer cell membrane surface was also confirmed using the anti-CAPRIN-1 antibody #1 obtained in Example 2.

Example 5

Preparation of Human-Mouse Chimeric Monoclonal Antibody

The gene amplification fragment comprising the nucleotide sequence of the heavy chain variable region of the anti-CAPRIN-1 antibody #1 obtained in Example 2 was treated at both ends with restriction enzymes, then purified, and inserted according to a routine method into a pcDNA4/myc-His vector (manufactured by Invitrogen Corp.) already having inserts of a mouse antibody-derived leader sequence and a human IgG_1 H chain constant region comprising the amino acid sequence of SEQ ID NO: 6. Also, the gene amplification fragment comprising the nucleotide sequence of the light chain variable region of the anti-CAPRIN-1 antibody #1 was treated at both ends with restriction enzymes, then purified, and inserted according to a routine method into a pcDNA3.1/myc-His vector (manufactured by Invitrogen Corp.) already having inserts of a mouse antibody-derived leader sequence and a human IgG_1 L chain constant region comprising the amino acid sequence of SEQ ID NO: 7.

Next, the recombinant vector having the insert of the heavy chain variable region of the anti-CAPRIN-1 antibody #1 and the recombinant vector having the insert of the light chain variable region were introduced into CHO-K1 cells (obtained from Riken Cell Bank). Specifically, 2×10^5 CHO-K1 cells were cultured in 1 ml of a Ham's F12 medium (manufactured by Invitrogen Corp.) containing 10% FBS per well of a 12-well culture plate, and washed with PBS(−). Then, 1 ml of a fresh Ham's F12 medium containing 10% FBS per well was added thereto. 250 ng each of the vectors dissolved in 30 μl of OptiMEM (manufactured by Invitrogen Corp.) was mixed with 30 μl of Polyfect transfection reagent (manufactured by Qiagen N.V.), and this mixture was added to each well. The CHO-K1 cells cotransfected with the recombinant vectors were cultured in a Ham's F12 medium containing 10% FBS supplemented with 200 μg/ml Zeocin (manufactured by Invitrogen Corp.) and 200 μg/ml Geneticin (manufactured by Roche Diagnostics K.K.) and then inoculated to a 96-well plate at 0.5 cells/well to prepare cell lines stably producing human-mouse chimeric monoclonal antibody #1 having the variable regions of the anti-CAPRIN-1 antibody #1 obtained in Example 2.

Each prepared cell line was cultured for 5 days in a 150-cm^2 flask at 5×10^5 cells/ml using 30 ml of a serum-free Opti-CHO medium (manufactured by Invitrogen Corp.) to obtain culture supernatants containing the human-mouse chimeric monoclonal antibody #1.

Also, cell lines stably producing human-mouse chimeric antibodies 1 to 26 were prepared as comparative samples in the same way as above respectively using the following comparative antibodies: anti-CAPRIN-1 mouse-derived monoclonal antibodies described in WO2010/016526, i.e., a comparative antibody 1 having the heavy chain variable region of SEQ ID NO: 26 (disclosed in WO2010/016526, the same holds true for the description below) and the light chain variable region of SEQ ID NO: 27, a comparative antibody 2 having the heavy chain variable region of SEQ ID NO: 28 and the light chain variable region of SEQ ID NO: 29, a comparative antibody 3 having the heavy chain variable region of SEQ ID NO: 30 and the light chain variable region of SEQ ID NO: 31, a comparative antibody 4 having the heavy chain variable region of SEQ ID NO: 32 and the light chain variable region of SEQ ID NO: 33, a comparative antibody 5 having the heavy chain variable region of SEQ ID NO: 34 and the light chain variable region of SEQ ID NO: 35, a comparative antibody 6 having the heavy chain variable region of SEQ ID NO: 36 and the light chain variable region of SEQ ID NO: 37, a comparative antibody 7 having the heavy chain variable region of SEQ ID NO: 38 and the light chain variable region of SEQ ID NO: 39, a comparative antibody 8 having the heavy chain variable region of SEQ ID NO: 40 and the light chain variable region of SEQ ID NO: 41, a comparative

antibody 9 having the heavy chain variable region of SEQ ID NO: 42 and the light chain variable region of SEQ ID NO: 43, a comparative antibody 10 having the heavy chain variable region of SEQ ID NO: 44 and the light chain variable region of SEQ ID NO: 45, and a comparative antibody 11 having the heavy chain variable region of SEQ ID NO: 46 and the light chain variable region of SEQ ID NO: 47; anti-CAPRIN-1 monoclonal antibodies described in WO2011/096517, i.e., a comparative antibody 12 having the heavy chain variable region of SEQ ID NO: 43 (disclosed in WO2011/096517; the same holds true for the description below) and the light chain variable region of SEQ ID NO: 47, and a comparative antibody 13 having the heavy chain variable region of SEQ ID NO: 43 and the light chain variable region of SEQ ID NO:; anti-CAPRIN-1 monoclonal antibodies described in WO2011/096528, i.e., a comparative antibody 14 having the heavy chain variable region of SEQ ID NO: 43 (disclosed in WO2011/096528; the same holds true for the description below) and the light chain variable region of SEQ ID NO: 47, a comparative antibody 15 having the heavy chain variable region of SEQ ID NO: 51 and the light chain variable region of SEQ ID NO: 55, a comparative antibody 16 having the heavy chain variable region of SEQ ID NO: 59 and the light chain variable region of SEQ ID NO: 63, a comparative antibody 17 having the heavy chain variable region of SEQ ID NO: 76 and the light chain variable region of SEQ ID NO: 80, a comparative antibody 18 having the heavy chain variable region of SEQ ID NO: 84 and the light chain variable region of SEQ ID NO: 88, and a comparative antibody 19 having the heavy chain variable region of SEQ ID NO: 92 and the light chain variable region of SEQ ID NO: 96; an anti-CAPRIN-1 monoclonal antibody described in WO2011/096519, i.e., a comparative antibody 20 having the heavy chain variable region of SEQ ID NO: 42 (disclosed in WO2011/096519; the same holds true for the description below) and the light chain variable region of SEQ ID NO: 46; anti-CAPRIN-1 monoclonal antibodies described in WO2011/096533, i.e., a comparative antibody 21 having the heavy chain variable region of SEQ ID NO: 43 (disclosed in WO2011/096533; the same holds true for the description below) and the light chain variable region of SEQ ID NO: 51, a comparative antibody 22 having the heavy chain variable region of SEQ ID NO: 47 and the light chain variable region of SEQ ID NO: 51, and a comparative antibody 23 having the heavy chain variable region of SEQ ID NO: 63 and the light chain variable region of SEQ ID NO: 67; and anti-CAPRIN-1 monoclonal antibodies described in WO2011/096534, a comparative antibody 24 having the heavy chain variable region of SEQ ID NO: 43 (described therein; the same holds true for the description below) and the light chain variable region of SEQ ID NO: 47, a comparative antibody 25 having the heavy chain variable region of SEQ ID NO: 43 and the light chain variable region of SEQ ID NO: 51, and a comparative antibody 26 having the heavy chain variable region of SEQ ID NO: 63 and the light chain variable region of SEQ ID NO: 67. Each prepared cell line was cultured for 5 days in a 150-cm^2 flask at 5×10^5 cells/ml using 30 ml of a serum-free OptiCHO medium (manufactured by Invitrogen Corp.) to obtain culture supernatants containing each of the human-mouse chimeric comparative monoclonal antibodies 1 to 26.

Example 6

Expression of CAPRIN-1 on Surface of Various Cancer Cells Using Anti-CAPRIN-1 Monoclonal Antibody

Next, the 8 human breast cancer cell lines (ZR75-1, MCF7, T47D, SK-BR-3, MDA-MB-157, BT-20, MDA-MB-231V, and MRK-nu-1), the 2 kidney cancer cell lines (Caki-1 and Caki-2), the urinary bladder cancer cell line (T24), the ovary cancer cell line (SKOV3), the 2 lung cancer cell lines (QG56 and A549), the prostate cancer cell line (PC3), the uterine cervix cancer cell line (SW756), the fibrosarcoma cell line (HT1080), the 2 brain tumor cell lines (T98G and U87MG), the gastric cancer cell line (MNK28), the 3 colorectal cancer cell lines (Lovo, DLD-1, and HCT-116), and the 4 pancreatic cancer cell lines (Capan-2, MIAPaCa-2, Panc-1, and BxPC-3) observed to have CAPRIN-1 gene expression were examined for their expression of CAPRIN-1 proteins on the cell surface using the culture supernatants containing the anti-CAPRIN-1 antibody #1 obtained in Example 2. 10^6 cells of each cell line were centrifuged in each 1.5-ml microcentrifuge tube. Each culture supernatant (100 μl) containing the antibody was added to the tube and left standing for 1 hour on ice. After washing with PBS, FITC-labeled goat anti-mouse IgG (H+L) antibodies (manufactured by Jackson ImmunoResearch Laboratories, Inc.) diluted with PBS containing 0.1% FBS were added thereto and left standing at 4° C. for 30 minutes. After washing with PBS, the fluorescence intensity was measured using FACSCalibur (Becton, Dickinson and Company). The negative control sample used was prepared by reacting the cell line only with secondary antibodies. As a result, the anti-CAPRIN-1 antibody #1 exhibited reactivity with fluorescence intensity at least 30% stronger than that of the negative control. This demonstrated that CAPRIN-1 proteins are expressed on the cell membrane surface of the human cancer cell lines above. The rate of enhancement in fluorescence intensity above was expressed as the rate of increase in mean fluorescence intensity (MFI) in each cell line and calculated according to the following formula:

Rate of increase in mean fluorescence intensity(Rate of enhancement in fluorescence intensity)(%)= ((MFI of cells reacted with the anti-CAPRIN-1 antibodies)−(Control MFI))/(Control MFI)×100.

Example 7

Antitumor Activity Against Cancer Cell of Antibody Against CAPRIN-1-Derived Peptide (SEQ ID NO: 5)

In order to evaluate each antibody against the CAPRIN-1-derived peptide (SEQ ID NO: 5) for the strength of its cytotoxicity against cancer cells expressing CAPRIN-1, ADCC activity was determined. The polyclonal antibodies against the peptide (SEQ ID NO: 5) prepared in Example 3 were used in this evaluation. Similar evaluation was conducted using polyclonal antibodies against other human CAPRIN-1-derived peptides (polyclonal antibodies against amino acid residues 50 to 98 in the amino acid sequence of SEQ ID NO: 2 of human CAPRIN-1 and polyclonal antibodies against amino acid residues 233 to 305, which were prepared in Example 3) as comparative antibodies, and the rabbit serum-derived control antibodies prepared in Example 3 as a negative control.

The human breast cancer cell line MDA-MB-231V, the human colorectal cancer cell line DLD-1, the human pancreatic cancer cell line Capan-2, and the human lung cancer cell line QG56 observed to have CAPRIN-1 expression were each collected at 10^6 cells into a 50-ml centrifuge tube, and 100 μCi of chromium 51 was then added thereto, followed by incubation at 37° C. for 2 hours. Then, the cells were washed three times with an RPMI1640 medium containing 10% fetal calf serum and added at 2×10^3 cells/well to a 96-well V-bottom plate. The polyclonal antibodies against the human CAPRIN-1-derived peptide (SEQ ID NO: 5) and two types of poly-

clonal antibodies against other human CAPRIN-1-derived peptides (polyclonal antibodies against amino acid residues 50 to 98 in SEQ ID NO: 2 of human CAPRIN-1 and polyclonal antibodies against amino acid residues 233 to 305) as described above were each added thereto at 1 μg/well. Lymphocytes separated from human peripheral blood according to a conventional method were further added thereto at 4×10^5 cells/well and cultured for 4 hours at 37° C., 5% CO_2. After the culture, the amount of chromium (Cr) 51 released from damaged cancer cells was measured in the culture supernatant to calculate the ADCC activity against the cancer cells of the polyclonal antibodies against each human CAPRIN-1-derived peptide. As a result, both of the polyclonal antibodies obtained by immunization with the partial human CAPRIN-1 peptides having the amino acid sequence of amino acid residues 50 to 98 or amino acid residues 233 to 305 of SEQ ID NO: 2 of human CAPRIN-1 had activity less than 8% against the human breast cancer cell line MDA-MB-231V, the human colorectal cancer cell line DLD-1, the human pancreatic cancer cell line Capan-2, and the human lung cancer cell line QG56. By contrast, the groups administered with the polyclonal antibodies against the human CAPRIN-1-derived peptide (SEQ ID NO: 5) were observed to have 27% or higher cytotoxic activity for all the cancer cell lines. The negative control antibodies had activity less than 5% for all the cancer cells. These results demonstrated that the antibody against CAPRIN-1 shown in SEQ ID NO: 5 exerts strong cytotoxic activity against cancer cells expressing CAPRIN-1.

These results about cytotoxic activity were obtained by, as described above, mixing the antibody against CAPRIN-1 used in the present invention, lymphocytes, and 2×10^3 cells of each cancer cell line with incorporated chromium 51; culturing the cells for 4 hours; after the culture, measuring the amount of chromium 51 released into the medium; and calculating the cytotoxic activity against cancer cell line according to the following formula*:

Cytotoxic activity (%)=Amount of chromium 51 released from the target cells treated with the antibody against CAPRIN-1 and lymphocytes/Amount of chromium 51 released from target cells treated with 1N hydrochloric acid×100. *Formula:

The human-mouse chimeric monoclonal antibody obtained in Example 5 were evaluated for their cytotoxic activity against human cancer cells. The culture supernatant of each cell line producing the antibodies was purified using Hitrap Protein A Sepharose FF (manufactured by GE Healthcare Bio-Sciences Ltd.). After medium replacement with PBS (−), the solution was filtered through a 0.22-μm filter (manufactured by Millipore Corp.). The resulting antibody was used for activity assay. The human breast cancer cell line MDA-MB-231V, the human colorectal cancer cell line DLD-1, the human pancreatic cancer cell line Capan-2, and the human lung cancer cell line QG56 were each collected at 10^6 cells into a 50-ml centrifuge tube, and 100 μCi of chromium 51 was then added thereto, followed by incubation at 37° C. for 2 hours. Then, the cells were washed three times with an RPMI1640 medium containing 10% FBS and added at 2×10^3 cells/well to 96-well V-bottom plate to prepare target cells. The purified antibodies (human-mouse chimeric anti-CAPRIN-1 antibody #1 and the human-mouse chimeric comparative monoclonal antibodies 1 to 26 obtained in Example 5) were each added thereto at 0.75 μg/well. A cell population containing human NK cells was separated using a conventional method from human peripheral blood lymphocytes prepared according to a conventional method. Specifically, the cell population containing human NK cells used was prepared as follows: human peripheral blood mononuclear cells separated using a specific gravity separation solution Histopaque for peripheral blood mononuclear cell separation (Sigma-Aldrich Corp.) were reacted with FITC fluorescent dye-labeled antibodies (anti-human CD3 antibody, anti-human CD20 antibody, anti-human CD19 antibody, anti-human CD11c antibody, or anti-HLA-DR antibody (Becton, and Dickinson and Company)), and a cell population containing NK cells unstained with the antibodies was separated using a cell sorter (FACS Vantage SE (Becton, and Dickinson and Company)). Alternatively, a cell population containing human NK cells was separated with human NK cell separation kit (manufactured by Miltenyi Biotec K.K.). The separated cell population containing NK cells was added to the plate at 2×10^5 cells/well and cultured for 4 hours at 37° C., 5% CO_2. After the culture, the amount of chromium 51 released from damaged tumor cells was measured in the culture supernatant to calculate the cytotoxic activity of the anti-CAPRIN-1 antibodies against the cancer cells. The negative control sample used was prepared with isotype control antibodies. As a result, the isotype control antibodies used had cytotoxic activity of less than 5% for all of the cancer cell lines, and the human-mouse chimeric comparative monoclonal antibodies 1 to 26 used had cytotoxic activity of less than 5% against MDA-MB-231V, less than 8% against DLD-1, less than 6% against Capan-2, and less than 6% against QG56. By contrast, the human-mouse chimeric anti-CAPRIN-1 antibody #1 had cytotoxic activity of 14% or higher against MDA-MB-231V, 21% or higher against DLD-1, 27% or higher against Capan-2, and 20% or higher against QG56. Likewise, the isotype control antibodies used and the comparative antibodies 1 to 26 used had cytotoxic activity less than 4% against all of other cancer cells, i.e., breast cancer cell lines T47D, Hs578T, BT-20, SK-BR-3, MCF7, and MRK-nu-1, a glioma cell line T98G, a lung cancer cell line A549, a kidney cancer cell line Caki-1, a uterine cervix cancer cell line SW756, a urinary bladder cancer cell line T24, a gastric cancer cell line MKN28, a colorectal cancer cell line SW480, a leukemia cell line AML5, and a lymphoma cell line Ramos. By contrast, the human-mouse chimeric monoclonal antibodies were observed to have 10% or higher cytotoxic activity against these cell lines. These results showed that the antibodies against the CAPRIN-1-derived peptide shown in SEQ ID NO: 5 damage CAPRIN-1-expressing cancer cells through their ADCC activity, and demonstrated that the human-mouse chimeric anti-CAPRIN-1 antibody #1 exhibit stronger cytotoxic activity against human cancer cells than that of the comparative antibodies 1 to 26.

These results about cytotoxic activity were obtained by, as described above, mixing the antibody against CAPRIN-1 used in the present invention, lymphocytes (cell population containing NK cells), and 2×10^3 cells of each cancer cell line with incorporated chromium 51; culturing the cells for 4 hours; after the culture, measuring the amount of chromium 51 released into the medium; and calculating the cytotoxic activity against cancer cell line according to the following formula*:

Cytotoxic activity(%)=Amount of chromium 51 released from the target cells treated with the antibody against CAPRIN-1 and lymphocytes (cell population containing NK cells)/Amount of chromium 51 released from target cells treated with 1N hydrochloric acid×100. *Formula:

Example 8

The Number of CAPRIN-1 Molecules on Surface of Various Cancer Cells Recognized by Anti-CAPRIN-1 Antibody #1

A human breast cancer cell line (MDA-MB-231V), a kidney cancer cell line (Caki-1), a urinary bladder cancer cell line

(T24), an ovary cancer cell line (SKOV3), lung cancer cell lines (QG56 and A549), a pancreatic cancer cell line (Capan-2), a prostate cancer cell line (PC3), a uterine cervix cancer cell line (SW756), a fibrosarcoma cell line (HT1080), a brain tumor cell line (T98G), a gastric cancer cell line (MKN28), colorectal cancer cell lines (Lovo and DLD-1), a leukemia cell line (AML5), and a lymphoma cell line (Ramos) were examined for the number of CAPRIN-1 molecules on their cell surface recognized by the anti-CAPRIN-1 antibody #1 using an molecular numbers assay kit "QIFIKIT" (manufactured by Dako Japan Inc.). Similarly, the number of CAPRIN-1 molecules on the surface of the various cancer cells was also examined using the anti-CAPRIN-1 comparative monoclonal antibodies 1 to 26 prepared in Example 5.

According to the protocol attached to the kit, each antibody (anti-CAPRIN-1 antibodies #1 or any of the comparative antibodies 1 to 26) was diluted into 5 μg/ml (final concentration) with PBS, and this dilution was added to each cell line and reacted for 30 minutes. After washing with PBS, fluorescently labeled secondary anti-mouse IgG antibodies attached to the kit were added, together with calibration beads attached to the kit, to each cell line and left standing for 45 minutes on ice. Each cell line and the calibration beads were washed with PBS. Then, the fluorescence intensity was measured using FACSCalibur (Becton, Dickinson and Company) to obtain a mean fluorescence intensity value (mean). Also, a mean fluorescence intensity value (mean) was obtained by the same assay as above for the comparative antibodies. The negative control sample used was prepared with isotype control antibodies, and a mean was also obtained. Each mean fluorescence intensity value (mean) was used to calculate the number of molecules according to the protocol attached to the kit. As a result, the numbers of CAPRIN-1 molecules on the surface of the various cancer cells recognized by the anti-CAPRIN-1 monoclonal antibody #1 and the comparative antibodies 12 to 26 were 10^5 or more per cell for all the examined human cancer cell lines. On the other hand, the numbers of molecules recognized by the comparative antibodies 1 to 11 was less than 10^5 per cell.

INDUSTRIAL APPLICABILITY

The antibody of the present invention is useful for the treatment and/or prevention of cancer.

All publications, patents, and patent applications cited herein are incorporated herein by reference in their entirety.

SEQUENCE LISTING

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<212> TYPE: DNA
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<400> SEQUENCE: 1

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gcgcgcacg atg ccc tcg gcc acc agc cac agc ggg agc ggc agc aag tcg     231
          Met Pro Ser Ala Thr Ser His Ser Gly Ser Gly Ser Lys Ser
          1               5                   10

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ggc gct gtc cag acc gag gcc atg aag cag att ctc ggg gtg atc gac      375
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Lys Lys Leu Arg Asn Leu Glu Lys Lys Lys Gly Lys Leu Asp Asp Tyr
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Gln Glu Arg Met Asn Lys Gly Glu Arg Leu Asn Gln Asp Gln Leu Asp
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Ala Val Ser Lys Tyr Gln Glu Val Thr Asn Asn Leu Glu Phe Ala Lys
95                  100                 105                 110

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Ile Lys Lys Thr Ala Arg Arg Glu Gln Leu Met Arg Glu Glu Ala Glu
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Arg Val Phe Gln Ser Asn Tyr Phe Asp Ser Thr His Asn His Gln Asn
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Gly Leu Cys Glu Glu Glu Glu Ala Ala Ser Ala Pro Ala Val Glu Asp
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Gln Val Pro Glu Ala Glu Pro Glu Pro Ala Glu Glu Tyr Thr Glu Gln
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Gln Thr Leu Asp Pro Ala Ile Val Ser Ala Gln Pro Met Asn Pro Thr
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tcattttatg tttaagctcc tgaatctgca ttccacttgg gttgttttta agcattctaa 5349

attttagttg attataagtt agatttcaca gaatcagtat tgcccttgat cttgtccttt 5409

ttatggagtt aacggggagg aagacccctc aggaaaacga aagtaaattg ttaaggctca 5469

tcttcatacc tttttccatt ttgaatccta caaaaatact gcaaaagact agtgaatgtt 5529

taaaattaca ctagattaaa taatatgaaa gtc 5562

<210> SEQ ID NO 2
<211> LENGTH: 709
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 2

Met Pro Ser Ala Thr Ser His Ser Gly Ser Gly Ser Lys Ser Ser Gly
1 5 10 15

Pro Pro Pro Pro Ser Gly Ser Ser Gly Ser Glu Ala Ala Ala Gly Ala
20 25 30

Gly Ala Ala Ala Pro Ala Ser Gln His Pro Ala Thr Gly Thr Gly Ala
35 40 45

Val Gln Thr Glu Ala Met Lys Gln Ile Leu Gly Val Ile Asp Lys Lys
50 55 60

Leu Arg Asn Leu Glu Lys Lys Lys Gly Lys Leu Asp Asp Tyr Gln Glu
65 70 75 80

Arg Met Asn Lys Gly Glu Arg Leu Asn Gln Asp Gln Leu Asp Ala Val
85 90 95

Ser Lys Tyr Gln Glu Val Thr Asn Asn Leu Glu Phe Ala Lys Glu Leu
100 105 110

Gln Arg Ser Phe Met Ala Leu Ser Gln Asp Ile Gln Lys Thr Ile Lys
115 120 125

Lys Thr Ala Arg Arg Glu Gln Leu Met Arg Glu Glu Ala Glu Gln Lys
130 135 140

Arg Leu Lys Thr Val Leu Glu Leu Gln Tyr Val Leu Asp Lys Leu Gly
145 150 155 160

Asp Asp Glu Val Arg Thr Asp Leu Lys Gln Gly Leu Asn Gly Val Pro
165 170 175

Ile Leu Ser Glu Glu Glu Leu Ser Leu Leu Asp Glu Phe Tyr Lys Leu
180 185 190

Val Asp Pro Glu Arg Asp Met Ser Leu Arg Leu Asn Glu Gln Tyr Glu
195 200 205

His Ala Ser Ile His Leu Trp Asp Leu Leu Glu Gly Lys Glu Lys Pro
210 215 220

Val Cys Gly Thr Thr Tyr Lys Val Leu Lys Glu Ile Val Glu Arg Val
225 230 235 240

Phe Gln Ser Asn Tyr Phe Asp Ser Thr His Asn His Gln Asn Gly Leu
245 250 255

Cys Glu Glu Glu Glu Ala Ala Ser Ala Pro Ala Val Glu Asp Gln Val

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           260                 265                 270

Pro Glu Ala Glu Pro Glu Pro Ala Glu Glu Tyr Thr Glu Gln Ser Glu
        275                 280                 285

Val Glu Ser Thr Glu Tyr Val Asn Arg Gln Phe Met Ala Glu Thr Gln
    290                 295                 300

Phe Thr Ser Gly Glu Lys Glu Gln Val Asp Glu Trp Thr Val Glu Thr
305                 310                 315                 320

Val Glu Val Val Asn Ser Leu Gln Gln Gln Pro Gln Ala Ala Ser Pro
                325                 330                 335

Ser Val Pro Glu Pro His Ser Leu Thr Pro Val Ala Gln Ala Asp Pro
            340                 345                 350

Leu Val Arg Arg Gln Arg Val Gln Asp Leu Met Ala Gln Met Gln Gly
        355                 360                 365

Pro Tyr Asn Phe Ile Gln Asp Ser Met Leu Asp Phe Glu Asn Gln Thr
    370                 375                 380

Leu Asp Pro Ala Ile Val Ser Ala Gln Pro Met Asn Pro Thr Gln Asn
385                 390                 395                 400

Met Asp Met Pro Gln Leu Val Cys Pro Pro Val His Ser Glu Ser Arg
                405                 410                 415

Leu Ala Gln Pro Asn Gln Val Pro Val Gln Pro Glu Ala Thr Gln Val
            420                 425                 430

Pro Leu Val Ser Ser Thr Ser Glu Gly Tyr Thr Ala Ser Gln Pro Leu
        435                 440                 445

Tyr Gln Pro Ser His Ala Thr Glu Gln Arg Pro Gln Lys Glu Pro Ile
    450                 455                 460

Asp Gln Ile Gln Ala Thr Ile Ser Leu Asn Thr Asp Gln Thr Thr Ala
465                 470                 475                 480

Ser Ser Ser Leu Pro Ala Ala Ser Gln Pro Gln Val Phe Gln Ala Gly
                485                 490                 495

Thr Ser Lys Pro Leu His Ser Ser Gly Ile Asn Val Asn Ala Ala Pro
            500                 505                 510

Phe Gln Ser Met Gln Thr Val Phe Asn Met Asn Ala Pro Val Pro Pro
        515                 520                 525

Val Asn Glu Pro Glu Thr Leu Lys Gln Gln Asn Gln Tyr Gln Ala Ser
    530                 535                 540

Tyr Asn Gln Ser Phe Ser Ser Gln Pro His Gln Val Glu Gln Thr Glu
545                 550                 555                 560

Leu Gln Gln Glu Gln Leu Gln Thr Val Val Gly Thr Tyr His Gly Ser
                565                 570                 575

Pro Asp Gln Ser His Gln Val Thr Gly Asn His Gln Gln Pro Pro Gln
            580                 585                 590

Gln Asn Thr Gly Phe Pro Arg Ser Asn Gln Pro Tyr Tyr Asn Ser Arg
        595                 600                 605

Gly Val Ser Arg Gly Gly Ser Arg Gly Ala Arg Gly Leu Met Asn Gly
    610                 615                 620

Tyr Arg Gly Pro Ala Asn Gly Phe Arg Gly Gly Tyr Asp Gly Tyr Arg
625                 630                 635                 640

Pro Ser Phe Ser Asn Thr Pro Asn Ser Gly Tyr Thr Gln Ser Gln Phe
                645                 650                 655

Ser Ala Pro Arg Asp Tyr Ser Gly Tyr Gln Arg Asp Gly Tyr Gln Gln
            660                 665                 670

Asn Phe Lys Arg Gly Ser Gly Gln Ser Gly Pro Arg Gly Ala Pro Arg
        675                 680                 685
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Gly Arg Gly Gly Pro Pro Arg Pro Asn Arg Gly Met Pro Gln Met Asn
    690                 695                 700

Thr Gln Gln Val Asn
705


<210> SEQ ID NO 3
<211> LENGTH: 3553
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (190)..(2274)
<223> OTHER INFORMATION:

<400> SEQUENCE: 3

cagagggctg ctggctggct aagtccctcc cgctcccggc tctcgcctca ctaggagcgg     60

ctctcggtgc agcgggacag ggcgaagcgg cctgcgccca cggagcgcgc gacactgccc    120

ggaagggacc gccacccttg ccccctcagc tgcccactcg tgatttccag cggcctccgc    180

gcgcgcacg atg ccc tcg gcc acc agc cac agc ggg agc ggc agc aag tcg    231
          Met Pro Ser Ala Thr Ser His Ser Gly Ser Gly Ser Lys Ser
          1               5                   10

tcc gga ccg cca ccg ccg tcg ggt tcc tcc ggg agt gag gcg gcc gcg     279
Ser Gly Pro Pro Pro Pro Ser Gly Ser Ser Gly Ser Glu Ala Ala Ala
15                  20                  25                  30

gga gcc ggg gcc gcc gcg ccg gct tct cag cac ccc gca acc ggc acc     327
Gly Ala Gly Ala Ala Ala Pro Ala Ser Gln His Pro Ala Thr Gly Thr
                35                  40                  45

ggc gct gtc cag acc gag gcc atg aag cag att ctc ggg gtg atc gac     375
Gly Ala Val Gln Thr Glu Ala Met Lys Gln Ile Leu Gly Val Ile Asp
            50                  55                  60

aag aaa ctt cgg aac ctg gag aag aaa aag ggt aag ctt gat gat tac     423
Lys Lys Leu Arg Asn Leu Glu Lys Lys Lys Gly Lys Leu Asp Asp Tyr
        65                  70                  75

cag gaa cga atg aac aaa ggg gaa agg ctt aat caa gat cag ctg gat     471
Gln Glu Arg Met Asn Lys Gly Glu Arg Leu Asn Gln Asp Gln Leu Asp
    80                  85                  90

gcc gtt tct aag tac cag gaa gtc aca aat aat ttg gag ttt gca aaa     519
Ala Val Ser Lys Tyr Gln Glu Val Thr Asn Asn Leu Glu Phe Ala Lys
95                  100                 105                 110

gaa tta cag agg agt ttc atg gca cta agt caa gat att cag aaa aca     567
Glu Leu Gln Arg Ser Phe Met Ala Leu Ser Gln Asp Ile Gln Lys Thr
                115                 120                 125

ata aag aag aca gca cgt cgg gag cag ctt atg aga gaa gaa gct gaa     615
Ile Lys Lys Thr Ala Arg Arg Glu Gln Leu Met Arg Glu Glu Ala Glu
            130                 135                 140

cag aaa cgt tta aaa act gta ctt gag cta cag tat gtt ttg gac aaa     663
Gln Lys Arg Leu Lys Thr Val Leu Glu Leu Gln Tyr Val Leu Asp Lys
        145                 150                 155

ttg gga gat gat gaa gtg cgg act gac ctg aaa caa ggt ttg aat gga     711
Leu Gly Asp Asp Glu Val Arg Thr Asp Leu Lys Gln Gly Leu Asn Gly
    160                 165                 170

gtg cca ata ttg tcc gaa gag gag ttg tca ttg ttg gat gaa ttc tat     759
Val Pro Ile Leu Ser Glu Glu Glu Leu Ser Leu Leu Asp Glu Phe Tyr
175                 180                 185                 190

aag cta gta gac cct gaa cgg gac atg agc ttg agg ttg aat gaa cag     807
Lys Leu Val Asp Pro Glu Arg Asp Met Ser Leu Arg Leu Asn Glu Gln
                195                 200                 205

tat gaa cat gcc tcc att cac ctg tgg gac ctg ctg gaa ggg aag gaa     855
Tyr Glu His Ala Ser Ile His Leu Trp Asp Leu Leu Glu Gly Lys Glu
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           210                 215                 220

aaa cct gta tgt gga acc acc tat aaa gtt cta aag gaa att gtt gag      903
Lys Pro Val Cys Gly Thr Thr Tyr Lys Val Leu Lys Glu Ile Val Glu
        225                 230                 235

cgt gtt ttt cag tca aac tac ttt gac agc acc cac aac cac cag aat      951
Arg Val Phe Gln Ser Asn Tyr Phe Asp Ser Thr His Asn His Gln Asn
    240                 245                 250

ggg ctg tgt gag gaa gaa gag gca gcc tca gca cct gca gtt gaa gac      999
Gly Leu Cys Glu Glu Glu Glu Ala Ala Ser Ala Pro Ala Val Glu Asp
255                 260                 265                 270

cag gta cct gaa gct gaa cct gag cca gca gaa gag tac act gag caa     1047
Gln Val Pro Glu Ala Glu Pro Glu Pro Ala Glu Glu Tyr Thr Glu Gln
                275                 280                 285

agt gaa gtt gaa tca aca gag tat gta aat aga cag ttc atg gca gaa     1095
Ser Glu Val Glu Ser Thr Glu Tyr Val Asn Arg Gln Phe Met Ala Glu
            290                 295                 300

aca cag ttc acc agt ggt gaa aag gag cag gta gat gag tgg aca gtt     1143
Thr Gln Phe Thr Ser Gly Glu Lys Glu Gln Val Asp Glu Trp Thr Val
        305                 310                 315

gaa acg gtt gag gtg gta aat tca ctc cag cag caa cct cag gct gca     1191
Glu Thr Val Glu Val Val Asn Ser Leu Gln Gln Gln Pro Gln Ala Ala
    320                 325                 330

tcc cct tca gta cca gag ccc cac tct ttg act cca gtg gct cag gca     1239
Ser Pro Ser Val Pro Glu Pro His Ser Leu Thr Pro Val Ala Gln Ala
335                 340                 345                 350

gat ccc ctt gtg aga aga cag cga gta caa gac ctt atg gca caa atg     1287
Asp Pro Leu Val Arg Arg Gln Arg Val Gln Asp Leu Met Ala Gln Met
                355                 360                 365

cag ggt ccc tat aat ttc ata cag gat tca atg ctg gat ttt gaa aat     1335
Gln Gly Pro Tyr Asn Phe Ile Gln Asp Ser Met Leu Asp Phe Glu Asn
            370                 375                 380

cag aca ctt gat cct gcc att gta tct gca cag cct atg aat cca aca     1383
Gln Thr Leu Asp Pro Ala Ile Val Ser Ala Gln Pro Met Asn Pro Thr
        385                 390                 395

caa aac atg gac atg ccc cag ctg gtt tgc cct cca gtt cat tct gaa     1431
Gln Asn Met Asp Met Pro Gln Leu Val Cys Pro Pro Val His Ser Glu
    400                 405                 410

tct aga ctt gct cag cct aat caa gtt cct gta caa cca gaa gcg aca     1479
Ser Arg Leu Ala Gln Pro Asn Gln Val Pro Val Gln Pro Glu Ala Thr
415                 420                 425                 430

cag gtt cct ttg gta tca tcc aca agt gag ggg tac aca gca tct caa     1527
Gln Val Pro Leu Val Ser Ser Thr Ser Glu Gly Tyr Thr Ala Ser Gln
                435                 440                 445

ccc ttg tac cag cct tct cat gct aca gag caa cga cca cag aag gaa     1575
Pro Leu Tyr Gln Pro Ser His Ala Thr Glu Gln Arg Pro Gln Lys Glu
            450                 455                 460

cca att gat cag att cag gca aca atc tct tta aat aca gac cag act     1623
Pro Ile Asp Gln Ile Gln Ala Thr Ile Ser Leu Asn Thr Asp Gln Thr
        465                 470                 475

aca gca tca tca tcc ctt cct gct gcg tct cag cct caa gta ttt cag     1671
Thr Ala Ser Ser Ser Leu Pro Ala Ala Ser Gln Pro Gln Val Phe Gln
    480                 485                 490

gct ggg aca agc aaa cct tta cat agc agt gga atc aat gta aat gca     1719
Ala Gly Thr Ser Lys Pro Leu His Ser Ser Gly Ile Asn Val Asn Ala
495                 500                 505                 510

gct cca ttc caa tcc atg caa acg gtg ttc aat atg aat gcc cca gtt     1767
Ala Pro Phe Gln Ser Met Gln Thr Val Phe Asn Met Asn Ala Pro Val
                515                 520                 525

cct cct gtt aat gaa cca gaa act tta aaa cag caa aat cag tac cag     1815
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Pro Pro Val Asn Glu Pro Glu Thr Leu Lys Gln Gln Asn Gln Tyr Gln
            530                 535                 540

gcc agt tat aac cag agc ttt tct agt cag cct cac caa gta gaa caa     1863
Ala Ser Tyr Asn Gln Ser Phe Ser Ser Gln Pro His Gln Val Glu Gln
        545                 550                 555

aca gag ctt cag caa gaa cag ctt caa aca gtg gtt ggc act tac cat     1911
Thr Glu Leu Gln Gln Glu Gln Leu Gln Thr Val Val Gly Thr Tyr His
    560                 565                 570

ggt tcc cca gac cag tcc cat caa gtg act ggt aac cac cag cag cct     1959
Gly Ser Pro Asp Gln Ser His Gln Val Thr Gly Asn His Gln Gln Pro
575                 580                 585                 590

cct cag cag aac act gga ttt cca cgt agc aat cag ccc tat tac aat     2007
Pro Gln Gln Asn Thr Gly Phe Pro Arg Ser Asn Gln Pro Tyr Tyr Asn
                595                 600                 605

agt cgt ggt gtg tct cgt gga ggc tcc cgt ggt gct aga ggc ttg atg     2055
Ser Arg Gly Val Ser Arg Gly Gly Ser Arg Gly Ala Arg Gly Leu Met
            610                 615                 620

aat gga tac cgg ggc cct gcc aat gga ttc aga gga gga tat gat ggt     2103
Asn Gly Tyr Arg Gly Pro Ala Asn Gly Phe Arg Gly Gly Tyr Asp Gly
        625                 630                 635

tac cgc cct tca ttc tct aac act cca aac agt ggt tat aca cag tct     2151
Tyr Arg Pro Ser Phe Ser Asn Thr Pro Asn Ser Gly Tyr Thr Gln Ser
    640                 645                 650

cag ttc agt gct ccc cgg gat tac tct ggc tat caa cgg gat gga tat     2199
Gln Phe Ser Ala Pro Arg Asp Tyr Ser Gly Tyr Gln Arg Asp Gly Tyr
655                 660                 665                 670

cag cag aat ttc aag cga ggc tct ggg cag agt gga cca cgg gga gcc     2247
Gln Gln Asn Phe Lys Arg Gly Ser Gly Gln Ser Gly Pro Arg Gly Ala
                675                 680                 685

cca cga ggt aat att ttg tgg tgg tga tcctagctcc taagtggagc           2294
Pro Arg Gly Asn Ile Leu Trp Trp
            690

ttctgttctg gccttggaag agctgttaat agtctgcatg ttaggaatac atttatcctt   2354

tccagacttg ttgctaggga ttaaatgaaa tgctctgttt ctaaaactta atcttggacc   2414

caaattttaa tttttgaatg atttaatttt ccctgttact atataaactg tcttgaaaac   2474

tagaacatat tctcttctca gaaaaagtgt ttttccaact gaaaattatt tttcaggtcc   2534

taaaacctgc taaatgtttt taggaagtac ttactgaaac atttttgtaa gacatttttg   2594

gaatgagatt gaacatttat ataaatttat tattcctctt tcatttttttt gaaacatgcc   2654

tattatattt tagggccaga cacccttttaa tggccggata agccatagtt aacatttaga   2714

gaaccattta gaagtgatag aactaatgga atttgcaatg ccttttggac ctctattagt   2774

gatataaata tcaagttatt tctgactttt aaacaaaact cccaaattcc taacttattg   2834

agctatactt aaaaaaaatt acaggtttag agagtttttt gtttttcttt tactgttgga   2894

aaactacttc ccattttggc aggaagttaa cctatttaac aattagagct agcatttcat   2954

gtagtctgaa attctaaatg gttctctgat ttgagggagg ttaaacatca aacaggtttc   3014

ctctattggc cataacatgt ataaaatgtg tgttaaggag gaattacaac gtactttgat   3074

ttgaatacta gtagaaactg gccaggaaaa aggtacattt ttctaaaaat taatggatca   3134

cttgggaatt actgacttga ctagaagtat caaaggatgt ttgcatgtga atgtgggtta   3194

tgttctttcc caccttgtag catattcgat gaaagttgag ttaactgata gctaaaaatc   3254

tgttttaaca gcatgtaaaa agttatttta tctgttaaaa gtcattatac agttttgaat   3314

gttatgtagt ttctttttaa cagtttaggt aataaggtct gttttcattc tggtgctttt   3374
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attaattttg atagtatgat gttacttact actgaaatgt aagctagagt gtacactaga 3434

atgtaagctc catgagagca ggtaccttgt ctgtcttctc tgctgtatct attcccaacg 3494

cttgatgatg gtgcctggca catagtaggc actcaataaa tatttgttga atgaatgaa 3553

<210> SEQ ID NO 4
<211> LENGTH: 694
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 4

Met Pro Ser Ala Thr Ser His Ser Gly Ser Gly Ser Lys Ser Ser Gly
1 5 10 15

Pro Pro Pro Pro Ser Gly Ser Ser Gly Ser Glu Ala Ala Ala Gly Ala
20 25 30

Gly Ala Ala Ala Pro Ala Ser Gln His Pro Ala Thr Gly Thr Gly Ala
35 40 45

Val Gln Thr Glu Ala Met Lys Gln Ile Leu Gly Val Ile Asp Lys Lys
50 55 60

Leu Arg Asn Leu Glu Lys Lys Lys Gly Lys Leu Asp Asp Tyr Gln Glu
65 70 75 80

Arg Met Asn Lys Gly Glu Arg Leu Asn Gln Asp Gln Leu Asp Ala Val
85 90 95

Ser Lys Tyr Gln Glu Val Thr Asn Asn Leu Glu Phe Ala Lys Glu Leu
100 105 110

Gln Arg Ser Phe Met Ala Leu Ser Gln Asp Ile Gln Lys Thr Ile Lys
115 120 125

Lys Thr Ala Arg Arg Glu Gln Leu Met Arg Glu Glu Ala Glu Gln Lys
130 135 140

Arg Leu Lys Thr Val Leu Glu Leu Gln Tyr Val Leu Asp Lys Leu Gly
145 150 155 160

Asp Asp Glu Val Arg Thr Asp Leu Lys Gln Gly Leu Asn Gly Val Pro
165 170 175

Ile Leu Ser Glu Glu Glu Leu Ser Leu Leu Asp Glu Phe Tyr Lys Leu
180 185 190

Val Asp Pro Glu Arg Asp Met Ser Leu Arg Leu Asn Glu Gln Tyr Glu
195 200 205

His Ala Ser Ile His Leu Trp Asp Leu Leu Glu Gly Lys Glu Lys Pro
210 215 220

Val Cys Gly Thr Thr Tyr Lys Val Leu Lys Glu Ile Val Glu Arg Val
225 230 235 240

Phe Gln Ser Asn Tyr Phe Asp Ser Thr His Asn His Gln Asn Gly Leu
245 250 255

Cys Glu Glu Glu Glu Ala Ala Ser Ala Pro Ala Val Glu Asp Gln Val
260 265 270

Pro Glu Ala Glu Pro Glu Pro Ala Glu Glu Tyr Thr Glu Gln Ser Glu
275 280 285

Val Glu Ser Thr Glu Tyr Val Asn Arg Gln Phe Met Ala Glu Thr Gln
290 295 300

Phe Thr Ser Gly Glu Lys Glu Gln Val Asp Glu Trp Thr Val Glu Thr
305 310 315 320

Val Glu Val Val Asn Ser Leu Gln Gln Gln Pro Gln Ala Ala Ser Pro
325 330 335

Ser Val Pro Glu Pro His Ser Leu Thr Pro Val Ala Gln Ala Asp Pro
340 345 350

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Leu Val Arg Arg Gln Arg Val Gln Asp Leu Met Ala Gln Met Gln Gly
        355                 360                 365

Pro Tyr Asn Phe Ile Gln Asp Ser Met Leu Asp Phe Glu Asn Gln Thr
    370                 375                 380

Leu Asp Pro Ala Ile Val Ser Ala Gln Pro Met Asn Pro Thr Gln Asn
385                 390                 395                 400

Met Asp Met Pro Gln Leu Val Cys Pro Pro Val His Ser Glu Ser Arg
                405                 410                 415

Leu Ala Gln Pro Asn Gln Val Pro Val Gln Pro Glu Ala Thr Gln Val
            420                 425                 430

Pro Leu Val Ser Ser Thr Ser Glu Gly Tyr Thr Ala Ser Gln Pro Leu
        435                 440                 445

Tyr Gln Pro Ser His Ala Thr Glu Gln Arg Pro Gln Lys Glu Pro Ile
    450                 455                 460

Asp Gln Ile Gln Ala Thr Ile Ser Leu Asn Thr Asp Gln Thr Thr Ala
465                 470                 475                 480

Ser Ser Ser Leu Pro Ala Ala Ser Gln Pro Gln Val Phe Gln Ala Gly
                485                 490                 495

Thr Ser Lys Pro Leu His Ser Ser Gly Ile Asn Val Asn Ala Ala Pro
            500                 505                 510

Phe Gln Ser Met Gln Thr Val Phe Asn Met Asn Ala Pro Val Pro Pro
        515                 520                 525

Val Asn Glu Pro Glu Thr Leu Lys Gln Gln Asn Gln Tyr Gln Ala Ser
    530                 535                 540

Tyr Asn Gln Ser Phe Ser Ser Gln Pro His Gln Val Glu Gln Thr Glu
545                 550                 555                 560

Leu Gln Gln Glu Gln Leu Gln Thr Val Val Gly Thr Tyr His Gly Ser
                565                 570                 575

Pro Asp Gln Ser His Gln Val Thr Gly Asn His Gln Gln Pro Pro Gln
            580                 585                 590

Gln Asn Thr Gly Phe Pro Arg Ser Asn Gln Pro Tyr Tyr Asn Ser Arg
        595                 600                 605

Gly Val Ser Arg Gly Gly Ser Arg Gly Ala Arg Gly Leu Met Asn Gly
    610                 615                 620

Tyr Arg Gly Pro Ala Asn Gly Phe Arg Gly Gly Tyr Asp Gly Tyr Arg
625                 630                 635                 640

Pro Ser Phe Ser Asn Thr Pro Asn Ser Gly Tyr Thr Gln Ser Gln Phe
                645                 650                 655

Ser Ala Pro Arg Asp Tyr Ser Gly Tyr Gln Arg Asp Gly Tyr Gln Gln
            660                 665                 670

Asn Phe Lys Arg Gly Ser Gly Gln Ser Gly Pro Arg Gly Ala Pro Arg
        675                 680                 685

Gly Asn Ile Leu Trp Trp
    690


<210> SEQ ID NO 5
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 5

Val Glu Arg Val Phe Gln Ser Asn Tyr Phe Asp Ser Thr His Asn His
1               5                   10                  15
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<210> SEQ ID NO 6
<211> LENGTH: 330
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 6

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1 5 10 15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65 70 75 80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
100 105 110

Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
115 120 125

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
130 135 140

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
145 150 155 160

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
165 170 175

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
180 185 190

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
195 200 205

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
210 215 220

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu
225 230 235 240

Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
245 250 255

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
260 265 270

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
275 280 285

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
290 295 300

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
305 310 315 320

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
325 330

<210> SEQ ID NO 7
<211> LENGTH: 106
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 7

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln

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1               5                   10                  15

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
            20                  25                  30

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
        35                  40                  45

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
    50                  55                  60

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
65                  70                  75                  80

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
                85                  90                  95

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
            100                 105


<210> SEQ ID NO 8
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 8

Asn Tyr Leu Ile Glu
1               5


<210> SEQ ID NO 9
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 9

Val Ile Asn Pro Lys Ser Gly Gly Thr Lys Tyr Asn Glu Lys Phe Arg
1               5                   10                  15

Gly Lys Ala


<210> SEQ ID NO 10
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 10

Thr Gly Thr Asp Tyr Trp Gly Gln Gly Thr Thr
1               5                   10


<210> SEQ ID NO 11
<211> LENGTH: 113
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 11

Gly Ala Glu Leu Val Arg Pro Gly Thr Ser Val Lys Val Ser Cys Lys
1               5                   10                  15

Ala Ser Val Tyr Ala Phe Thr Asn Tyr Leu Ile Glu Trp Val Lys Gln
            20                  25                  30

Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Val Ile Asn Pro Lys Ser
        35                  40                  45

Gly Gly Thr Lys Tyr Asn Glu Lys Phe Arg Gly Lys Ala Thr Leu Thr
    50                  55                  60

Ala Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr
65                  70                  75                  80

Ser Gly Asp Ser Ala Val Tyr Phe Cys Ala Ile Thr Gly Thr Asp Tyr
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                85                  90                  95

Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Ala Lys Thr Thr Pro
            100                 105                 110

Pro


<210> SEQ ID NO 12
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<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 12

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<210> SEQ ID NO 13
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 13

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1               5


<210> SEQ ID NO 14
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 14

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1               5


<210> SEQ ID NO 15
<211> LENGTH: 113
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 15

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Ser Ala Ser Leu Gly Asp Arg Val Thr Ile Ser Cys Ser Ala Ser Gln
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Gly Ile Asn Asn Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr
        35                  40                  45

Val Lys Leu Leu Ile Tyr Tyr Thr Ser Ser Leu Arg Ser Gly Val Pro
    50                  55                  60

Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile
65                  70                  75                  80

Ser Asn Leu Glu Pro Glu Asp Val Ala Thr Tyr Tyr Cys Gln Gln Tyr
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Ser Lys Leu Pro Arg Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
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Arg


<210> SEQ ID NO 16
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<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 16
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-continued

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gccttcacta attacttgat agagtgggta aagcagaggc ctggacaggg ccttgagtgg    120

attggagtga ttaatcctaa aagtggtggt actaagtaca atgagaagtt caggggcaag    180

gcaacactga ctgcagacaa atcctccagc actgcctaca tgcagctcag cagcctgaca    240

tctggtgact ctgcggtcta tttctgtgca ataactggga cagactactg gggccaaggc    300

accactctca cagtctcctc agccaaaaca acaccccca                           339


<210> SEQ ID NO 17
<211> LENGTH: 339
<212> TYPE: DNA
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<400> SEQUENCE: 17

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ggcgacagag tcaccatcag ttgcagtgca agtcagggca ttaacaatta tttaaactgg    120

tatcagcaaa aaccagatgg aactgttaaa ctcctgatct attacacatc aagtttacgc    180

tcaggagtcc catcaaggtt cagtggcagt gggtctggga cagattattc tctcaccatc    240

agcaacctgg aacctgaaga tgttgccact tactattgtc agcagtatag taagcttcct    300

cggacgttcg gtggcggcac caagctggaa atcaaacgg                           339
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The invention claimed is:

1. An antibody or a fragment thereof which has immunological reactivity with a partial CAPRIN-1 polypeptide consisting of the amino acid sequence set forth in SEQ ID NO: 5.

2. The antibody or fragment thereof according to claim **1**, wherein the antibody or fragment thereof has cytotoxic activity against a cancer cell expressing a CAPRIN-1 protein.

3. The antibody or fragment thereof according to claim **1**, wherein the antibody is a monoclonal antibody or a polyclonal antibody.

4. The antibody or fragment thereof according to claim **1**, wherein the antibody is a human antibody, a humanized antibody, a chimeric antibody, a single-chain antibody, or a multispecific antibody.

5. The antibody or fragment thereof according to claim **1**, wherein the antibody or fragment thereof comprises a heavy chain variable region comprising complementarity determining regions of SEQ ID NOs: 8, 9, and 10 (CDR1, CDR2, and CDR3, respectively) and a light chain variable region comprising complementarity determining regions of SEQ ID NOs: 12, 13, and 14 (CDR1, CDR2, and CDR3, respectively) and has immunological reactivity with the CAPRIN-1 protein.

6. The antibody or fragment thereof according to claim **1**, wherein the antibody or fragment thereof is conjugated with an antitumor agent.

7. A pharmaceutical composition for treatment of cancer, comprising an antibody or fragment thereof according to claim **1** as an active ingredient.

8. The pharmaceutical composition according to claim **7**, wherein the cancer is breast cancer, kidney cancer, pancreatic cancer, colorectal cancer, lung cancer, brain tumor, gastric cancer, uterine cervix cancer, ovary cancer, prostate cancer, urinary bladder cancer, esophageal cancer, leukemia, lymphoma, fibrosarcoma, mastocytoma, or melanoma.

9. A pharmaceutical combination for treatment of cancer, comprising the pharmaceutical composition according to claim **7** and a pharmaceutical composition comprising an antitumor agent.

10. A DNA encoding the antibody or fragment thereof according to claim **1**.

11. A method for treating cancer, comprising administering the antibody or fragment thereof according to claim **1** to a subject, wherein the cancer is a CAPRIN-1 expressing cancer.

12. The antibody or fragment thereof according to claim **2**, wherein the antibody is a monoclonal antibody or a polyclonal antibody.

13. The antibody or fragment thereof according to claim **2**, wherein the antibody is a human antibody, a humanized antibody, a chimeric antibody, a single-chain antibody, or a multispecific antibody.

14. The antibody or fragment thereof according to claim **3**, wherein the antibody is a human antibody, a humanized antibody, a chimeric antibody, a single-chain antibody, or a multispecific antibody.

15. The antibody or fragment thereof according to claim **2**, wherein the antibody or fragment thereof comprises a heavy chain variable region comprising complementarity determining regions of SEQ ID NOs: 8, 9, and 10 (CDR1, CDR2, and CDR3, respectively) and a light chain variable region comprising complementarity determining regions of SEQ ID NOs: 12, 13, and 14 (CDR1, CDR2, and CDR3, respectively) and has immunological reactivity with the CAPRIN-1 protein.

16. The antibody or fragment thereof according to claim **3**, wherein the antibody or fragment thereof comprises a heavy chain variable region comprising complementarity determining regions of SEQ ID NOs: 8, 9, and 10 (CDR1, CDR2, and CDR3, respectively) and a light chain variable region comprising complementarity determining regions of SEQ ID NOs: 12, 13, and 14 (CDR1, CDR2, and CDR3, respectively) and has immunological reactivity with the CAPRIN-1 protein.

17. The antibody or fragment thereof according to claim **4**, wherein the antibody or fragment thereof comprises a heavy

chain variable region comprising complementarity determining regions of SEQ ID NOs: 8, 9, and 10 (CDR1, CDR2, and CDR3, respectively) and a light chain variable region comprising complementarity determining regions of SEQ ID NOs: 12, 13, and 14 (CDR1, CDR2, and CDR3, respectively) and has immunological reactivity with the CAPRIN-1 protein.

18. The antibody or fragment thereof according to claim **2**, wherein the antibody or fragment thereof is conjugated with an antitumor agent.

19. The antibody or fragment thereof according to claim **3**, wherein the antibody or fragment thereof is conjugated with an antitumor agent.

20. The antibody or fragment thereof according to claim **4**, wherein the antibody or fragment thereof is conjugated with an antitumor agent.

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